THE MICROSCOPICAL EXAMINATION
OF
FOODS AND DRUGS

BY THE SAME AUTHOR.

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THE

MICROSCOPICAL EXAMINATION
OF
FOODS AND DRUGS

A PRACTICAL INTRODUCTION TO THE
METHODS ADOPTED IN THE MICROSCOPICAL EXAMINATION
OF FOODS AND DRUGS, IN THE ENTIRE, CRUSHED
AND POWDERED STATES

BY

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LATE EXTERNAL EXAMINER IN MATERIA MEDICA AND PHARMACY TO THE
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PREFACE

TO

THE SECOND EDITION

In preparing the second edition of this work for the press, no change has been made in the method of treating the subject matter, and but little in the details of those substances that were dealt with in the first edition, with the exception of the chapter on the Fibres which, with the valuable assistance of Miss Agnes Borrowman, has been carefully revised.

Several additions have, however, been made. Saffron has been introduced as an example of part of a flower, almond as type of the oily seeds now much used as alimentary substances, and fennel fruit as a type of umbelliferous fruits. Liquorice, gentian, and calumba have been added to the section dealing with the roots; the first two are largely used in the powdered state and are frequently adulterated; calumba is an example of a fleshy root exhibiting certain well-marked characters.

A chapter has also been added on the more commonly recurring adulterants of powdered foods and drugs. While it is often true that, as soon as a novel method of sophistication has been exposed, its use is abandoned, nevertheless several adulterants appear to be in more or less constant use, and it was considered desirable to introduce these to the notice of the student. Finally, a brief general scheme of examination has been appended, which may prove useful in the investigation of an unknown powder.

H. G. GREENISH.

LONDON, *September* 1910.

PREFACE

TO

THE FIRST EDITION

Nearly half a century has elapsed since Dr. Arthur Hill Hassall, in his classical works on the detection of adulterations in food and medicine, strongly advocated the use of the microscope as a valuable aid to chemical analysis in the examination of a large variety of vegetable substances, an advocacy that he supported by the publication of a great number of analyses.

During this time considerable progress has been made in the detection of such adulteration by chemical analysis, and numerous valuable memoirs and excellent text-books replete with details and literary references have been published. That branch of the subject, however, to which Dr. Hassall particularly devoted his attention—viz. the examination by the microscope—has unfortunately quite failed to keep pace with the examination by chemical analysis. Such portions of the present manuals of analysis as are devoted to the use of the microscope provide instructions that are but meagre, descriptions that lack precision, and illustrations that are wanting in detail. And yet the microscope is capable of furnishing, with the expenditure of a minimum of material and also often of a minimum of time, information concerning the substances analysed that cannot be obtained by any other means. With much truth Hassall observes that ‘ applying the microscope to food, it appears that there is scarcely a vegetable article of consumption, not a liquid, which may not be distinguished by means of that instrument. Further, that all those adulterations of these articles which consist in the addition of other vegetable substances and which constitute by far the majority of the adulterations practised, may likewise be discovered and discriminated by the same means.'[1]

Since the publication of Hassall's works, much has been done by the State to protect the public from the frauds that were then frequently practised upon it. There exist at the present time a large number of analysts engaged, more or less regularly, in the examination of foods and drugs. To these, in particular, the ability to use the microscope for the purpose of detecting adulteration, or confirming results arrived at by other means, must be of paramount importance. Indeed, it is difficult to understand how any public analyst that is unable to make an expert use of the microscope can be competent to discharge his duties. Upon this point the Local Government Board, the authority entrusted with the framing of regulations regarding the appointment of public analysts, has pronounced a definite opinion by making competency in the use of the microscope a necessary qualification, and the Institute of Chemistry of Great Britain and Ireland has established an examination that conforms in this respect with the requirements of the Board.

The Pharmaceutical Society of Great Britain has also recognised the importance to the pharmacist of skill in the use of the microscope for identifying the nature and determining the quality of powdered drugs. It is now some years since the Society introduced into its curriculum of study a suitable course of training in the use of the microscope, and into its examinations a test of the ability to use the knowledge thus acquired. Investigations recently conducted by the Society at the request of the General Medical Council have also shown that the microscopical examination of powdered drugs yields results of far greater value in determining their identity and purity than such chemical data as the amount of ash yielded by them.

Before the microscopical examination of vegetable foods and drugs can be intelligently practised, a general knowledge of botany and a fairly sound and thorough acquaintance with botanical histology are absolutely necessary. It is as impossible for anyone to become a competent microscopist without such preliminary knowledge, as to become a competent analytical chemist without first acquiring a sound knowledge of the theory and principles of the science of chemistry. For this reason it appears to me that the training now given in the School of Pharmacy of the Pharmaceutical Society—comprising, as it does, a knowledge of the principles upon which the sciences of botany and chemistry rest as well as training in the application of the knowledge thus acquired—is admirably adapted to fit a man to become an expert not only in the microscopical, but also in the chemical examination of foods and drugs.

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[1] *Adulterations Detected*, 1857, p. 15.

While there exist a number of excellent text-books of botany to aid the student in acquiring a knowledge of vegetable histology, there does not exist an English text-book specially devoted to practical instruction in the methods of examining vegetable foods, drugs, and their powders. The botanical text-books are not suitable for this purpose. They deal with the structure of vegetable organs from a general point of view, and devote little or no attention to the details that distinguish the individual members of any one class from one another, nor are the methods they employ generally suitable for analytical purposes. It was with a view to supplying this want that I undertook the compilation of the present volume. In it I have endeavoured to introduce the student to the chief methods adopted in the examination of vegetable foods and drugs, entire, crushed, and finely powdered. The best, in fact the only suitable, means of doing this seemed to me by selecting certain typical examples and describing the means by which they may be examined. The well-known danger that is incurred in teaching by types I have endeavoured to avoid by including a number of other examples which I have treated more briefly; hence in the earlier sections, dealing with starches, stems, and leaves, a comparatively large number have been examined, or at least referred to, while in the succeeding sections the number has been less. The selection of the substances to be examined has been the subject of grave consideration, inasmuch as it appeared desirable to examine such as illustrated varying methods, offered important but varying features in their structure, and yet were of general interest and in more or less common use.

The subject matter has been divided into twelve sections, which have been so arranged that the student may begin with the simplest and proceed to the most complex, acquiring, as he proceeds, a knowledge of various tests and operations that are of more or less general use. Thus I have commenced with the starches, which require but little preparation, and have proceeded to the fruits, which commonly possess a complex structure, and to the roots, which present difficulties in their identification when powdered. In order to exhibit the great variety of forms that starch may assume I have described and illustrated a rather large number, including, for practical reasons, all the most important. In dealing with leaves I have selected bearberry as an example of a coriaceous leaf that requires soaking in water before it is cut, that is easily cut, and that allows of the epidermis being separated after digestion with caustic potash ; senna, as a type of papery leaves that are best softened by exposure to a moist atmosphere, that are best cut in packets, and that exhibit their epidermis after soaking in chloral hydrate ; tea, on account of its importance as well as on account of the remarkable sclerenchymatous idioblasts it contains ; buchu, because it contains mucilage, oil glands, and hesperidin ; belladonna, as an example of a solanaceous leaf with bicollateral bundles and sandy crystals ; henbane and stramonium, as important objects for comparison, and so on throughout all the substances examined.

In very few instances is the examination of any food or drug complete without the preparation of sections in various directions, the separation of the tissues by suitable means, and the examination of the powder ; but nevertheless I have not dealt with all these operations until senna leaves are reached, because experience has taught me that the student may at that point most advantageously commence the study of the powder. For this purpose senna leaves of known genuineness should be reduced to a powder that will pass through a No. 60 sieve ; this will be coarser than the powdered senna of commerce, and more easily examined.

Although I have endeavoured to deal with substances yielding important powders, I have refrained from any attempt to embrace all such as the analyst or pharmacist may be likely to meet with. To supply this latter want I have in conjunction with M. Eugène Collin published a series of memoirs in the ‘ Pharmaceutical Journal ’ which will shortly be reprinted in the form of an Anatomical Atlas. Such an atlas can, however, be intelligently used only by those who have had preliminary training in botanical histology and in the methods of examining drugs and powders. The present volume is intended to afford to the student who has already been grounded in botany, the instruction and advice necessary to enable him to undertake the examination of vegetable powders. In its preparation I have received much valuable assistance from Mr. C. Heslop, Demonstrator in Pharmaceutics (1901–2), and Mr. T. E. Wallis, Assistant Lecturer and Senior Demonstrator in Chemistry and Physics. To Mr. Heslop I am indebted for help in reproducing several of the illustrations, while figs. 56, 116, 117, 118, 119, and 161 were prepared from drawings made by Mr. Wallis during the examination of the several substances under my own supervision. I have also made full use of the literature of the subject, particularly of the publications of Meyer, Moeller, Schimper, Tschirch, Tschirch and Oesterle, and Vogl.

H. G. GREENISH.

LONDON, *June* 1903.

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INTRODUCTION

To the student who accepts this work as a guide to conduct him from a knowledge of the general structure of vegetable organs to the study of the anatomical details of foods, drugs, and their powders, I address the following observations.

In order to thoroughly understand the anatomy of any drug, it is necessary first to examine it with a lens, then to cut sections in different directions and through different parts; to disintegrate the tissues by suitable means, and compare these with the tissues observed in the sections; to examine the powdered drug and compare the tissues, cells, and cell contents observed with those that previous examination has disclosed, noting the changes that they have undergone, and seeking and utilising special reactions to render more conspicuous such as are not easily detected.

Most strongly must I insist upon the necessity of recording observations by means of sketches. This affords a most valuable training for the powers of observation, as it compels the observer to examine each object more minutely and more attentively than would otherwise be the case. Much time may, however, be lost by injudicious or indiscriminate sketching. Diagrammatic sketches under a low power should first be made with a view of indicating the positions and extents of the various tissues; of such diagrammatic sketches figs. 53, 71, 88, and 187 may serve as examples.

The details of these tissues should then be drawn on a scale large enough to allow of the necessary minutiæ being introduced. Figs. 60, 125 to 128, 154, 155, 157 to 159, and 160 are examples of such. All sketches should be drawn in pencil and corrected until accurate, for one cell accurately drawn is a valuable record, whereas a hundred cells inaccurately drawn can only mislead. A little difficulty may at first be experienced in discriminating between the important and the unimportant; I have therefore in a number of instances endeavoured to aid the student by indicating what should be sketched.

It may of course be perfectly possible, by means of atlases, descriptions, or keys, to identify an unknown powder, but the identity cannot be considered as satisfactorily established until the powder has been compared with the powder of the drug with which it has been identified.

The student is recommended to prepare his own powders from the entire drug, passing them through a No. 60 or No. 80 sieve. After these have been studied the finer commercial powders which present more difficulty may be examined.

Finally, let me impress upon him the fact that there is no royal road available. Facility in the microscopical examination of foods and drugs can be acquired only by study and practice; without these it is impossible to become an expert in the use of the microscope.

FOODS AND DRUGS

SECTION I

STARCH

INTRODUCTION

Starch is one of the most widely distributed of the cell contents of plants. It is found in all classes, with the exception of the Fungi, and is met with in different parts of the same plant. It may, under certain circumstances, be detected in the form of minute grains in the chloroplastids of the leaf or stem, from which organs it is transported, in soluble form, to other organs destined to receive it. In these it may either be temporarily deposited until required for the growth of particular parts of those organs, as is the case with the small starch grains formed in the epidermis of the linseed and other seeds, or it may be more permanently deposited in comparatively large quantity as a reserve material to supply the subsequent needs of the plant. Seeds, fruits, rhizomes, roots, and aërial stems form the principal reservoirs for the storage of reserve starch, the presence of which often renders them valuable as foodstuffs or as sources of commercial starch.

Careful examination has shown that the starch grains produced by a particular plant are remarkably constant in size, shape, and general characters, but it has also been shown that the starch grains of one plant often differ from those of other plants to such an extent as to render the two kinds easily distinguishable. Sometimes, too, the starch grains produced by the various species of a single genus or natural order, exhibit a remarkable general resemblance to one another, as is the case, for instance, with the starch of many species of Leguminosæ.

The careful study of the starch grains, and especially of those deposited as reserve starch, becomes therefore of primary importance, both as a means of identifying the source of the different commercial varieties of starch and of distinguishing various starch-containing drugs from one another. The detection of starch is also frequently of great value as constituting a means of determining the adulteration of the powder of a drug naturally free from starch with either starch itself or with a drug containing that substance.

The following, therefore, are the chief points to be borne in mind in the examination of starch:

(1) The means by which starch grains can be identified as such.

(2) The means by which the starches of different plants may be distinguished from one another.

Examination of Potato Starch

Mounting.—Put a small drop of water on a slide; take a little potato starch on the point of a knife and transfer it to the water; mix thoroughly with a mounted needle and carefully cover with a coverslip. This should be done by gradually lowering the coverslip by means of the needle, preventing it from slipping by holding the finger against the edge that rests upon the slide. Care should be taken to avoid undue pressure, which is liable to crush the grains. Both slide and coverslip must be scrupulously clean, and any excess of water should be removed by a strip of filter paper, which, for this purpose, is preferable to blotting paper. It is very desirable to take, in the first instance, a drop of water of about the right size, but this can be attained only by practice.

Strict cleanliness in the mounting of objects for microscopical examination cannot be too strongly insisted upon. Excess of the mounting medium, if not removed as directed, allows of the coverslip floating, and of the objects under examination moving; moreover, the liquid is very liable to find its way on to the upper surface of the coverslip and thence on to the front lens of the objective, in either case obscuring clear vision. Too much starch is also objectionable, as the grains then lie over one another and accurate observation becomes impossible.

The presence of one or two bubbles of air is not a matter of importance, but a large number of them should be avoided; if they have by accident found their way into the preparation, as they may do if the coverslip has not been carefully lowered, a fresh preparation should be made.

Shape.—Examine the slide, first with a low power ($\frac{2}{3}$ inch or $\frac{1}{2}$ inch), and then with a high power ($\frac{1}{6}$ inch). The starch consists of grains of variable size. They have an oval, ovate, or ellipsoidal outline; some are triangular or even obscurely quadrangular, with rounded angles, and resemble oyster shells in shape.

The outline alone, however, does not give a sufficient clue to the shape. This must be ascertained by making the grains roll so that the same grain may be viewed in different positions. Examine a slide under the low power, and, while it is under observation, gently touch the edge of the coverslip with a needle. This will usually produce sufficient movement to cause some of the grains to roll and thus exhibit their form. In the case of small starch grains, sufficient movement may be produced by bringing a drop of alcohol on the slide to the edge of the coverslip; the alcohol, as it mixes with the water, sets up currents that carry the grains with them. Potato starch, when made to roll, is seen to be distinctly, though not strongly, flattened.

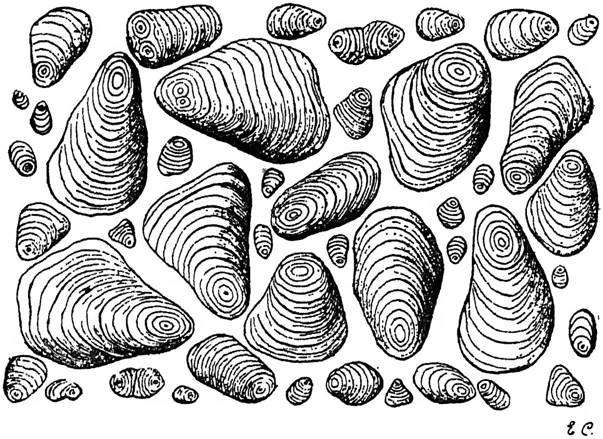

FIG. 1.—Potato Starch. × 240. (Greenish and Collin.)

Observe here and there a starch grain composed of two grains adhering by their broader (flattened) ends; such grains are termed 'compound.' Sometimes a compound grain is subsequently entirely surrounded by concentric rings of starch material; it is then called a 'semi-compound' grain.

Hilum.—Near one extremity (usually the narrower) there is a point around which concentric lines are arranged. This is the hilum. In some of the grains there is a small linear or **V**-shaped fissure through the hilum; this is often, but incorrectly, spoken of as the hilum—it is a fissure produced by the shrinkage attendant upon the drying of the grain.

Note that in potato starch the hilum is not in the centre of the grain; it is eccentric. It is often desirable to indicate the exact position of an eccentric hilum; this is done by measuring the distance between it and the nearer, as well as the further, margin of the grain and stating the ratio between these figures, which expresses the degree of eccentricity, as a fraction. Thus in potato starch the greater distance is about five times the lesser, and the eccentricity is therefore about $\frac{1}{5}$.

Striations.—Surrounding the hilum are a number of fine concentric lines; these are termed *striations* or *striæ*. They are said to be caused by variations in the amount of moisture present, the dark striations being comparatively rich in moisture, while the colourless intervening portions are comparatively poor; Meyer ascribes them to differences in the minute crystalline particles of which he believes the starch grain to consist. Observe that in potato starch there occur at intervals striations that are more strongly marked than the others.

The grains of many varieties of starch do not exhibit any striations at all; few exhibit them so conspicuously as potato starch does, and still fewer show some more and others less strongly marked. Faint striations may be made transiently more distinct by introducing under the coverslip a drop of solution of potash, or of chromic acid, or of chloral hydrate (compare p. 8, 'Effect of Caustic Alkali'); oblique illumination also aids in making them more easily visible.

Size.—The size of a starch grain, and of other microscopic objects, is usually ascertained by measurement with an ocular micrometer.

This instrument consists of a small glass circle on which an arbitrary scale is engraved. In the ordinary form this scale contains ten divisions, each of which is subdivided into ten subdivisions. The micrometer is introduced into the eyepiece by unscrewing the eye-lens and dropping it upon the diaphragm, the position of which has to be adjusted, so that the scale on the micrometer appears in focus on looking through the eyepiece.

The value of each division or subdivision of this scale has to be ascertained by means of a millimetre scale.

The millimetre scale, in its ordinary form, consists of a centimetre engraved upon a glass slide and divided into ten millimetres; one of the latter is subdivided into ten parts. The slide is focussed in the usual way, but using the eyepiece that contains the micrometer scale; the eyepiece must be rotated until the two scales are exactly superposed, when the number of divisions that correspond to, say, 1 millimetre on the millimetre scale can be read off. Thus, supposing 1 millimetre be exactly covered by 95 subdivisions of the ocular micrometer, then each subdivision of the latter will indicate $\frac{1}{95}$ or 0·0105 millimetre.

It is, however, usual to express measurements in terms, not of a millimetre, but of a micron (μ). A micron is the one-thousandth part of a millimetre, and, consequently, in the example quoted each subdivision of the scale will indicate 10·5 microns.

Having determined the value of the ocular micrometer scale as described, remove the slide with the millimetre scale from the stage of the microscope, and substitute for it the slide with potato starch grains. Bring the grain to be measured nearly into the centre of the field, and then rotate the eyepiece until the micrometer scale coincides with the long axis of the grain. The number of subdivisions the grain covers can then be easily read off and converted into microns by multiplying by the previously ascertained factor.

The value of the subdivisions of the micrometer will, of course, vary with the eyepiece and objective used. It will be found convenient to keep an eyepiece, with the micrometer fixed in it, specially for measuring, and to determine and record the value of the scale for each objective in general use.

In measuring starch grains in this way it is customary to measure them in water and to neglect the slight swelling that takes place when the dry grain is mounted in that liquid. In addition to the largest and smallest grain, those of most frequent occurrence should also be measured. It is sometimes desirable to ascertain the width as well as the length, but this is not often necessary.

Sketching.—Having thus carefully examined the starch, record the results by sketching a few of the grains. The importance of this as a means of training the power of observation, and ensuring that no detail has been overlooked, cannot be over-estimated. The student is urged on no account to neglect to sketch his preparations; although it may be a little troublesome at first, it will gradually become easier.

The simplest method of sketching is that of observing the object under the microscope and then reproducing it upon paper. In doing this care should be taken to sketch in pencil, and on a sufficiently large scale; the largest grains of potato starch, for instance, should measure not less than 2 cm. in length. Sketch first the outline, and make sure that it correctly represents the outline of the grain under examination. Next put in the hilum, taking care that this also is accurately done. Then count the number of darker striations, and sketch them in their correct relative positions. Lastly, count, if possible, the number of fainter striations between two dark striations, and introduce these. Two or three grains correctly sketched form a valuable record, but a dozen grains carelessly drawn are worse than useless. Typical grains should be selected, and the number not unnecessarily multiplied by repeated sketches of very similar grains. Focus as frequently as may be necessary in order to show all the details as sharply defined as possible.

It is exceedingly desirable that the grains should be reproduced in their correct relative size. This can easily be done by means of the ocular micrometer. Suppose, for instance, the potato starch is to be sketched under a magnification of 200 diameters. Measure the grain with the ocular micrometer. It measures, say, 100 μ ($=\frac{1}{10}$ mm.). It must be drawn, therefore, $200 \times \frac{1}{10}$ mm. ($=20$ mm.) long. Mark two points on the sketching paper this distance apart. Next, measure the distance of the hilum from the nearer margin and mark its position. Lastly, measure the breadth of the grain at its widest point, and mark this. The details can then be readily filled in. The second grain sketched can be treated in the same way. The exact magnification is known, and the relative size is correctly preserved.

More commonly, one of the various forms of camera lucida is used. Information concerning these can be obtained from one of the numerous works that deal specially with the microscope and its appliances, or from the price lists of manufacturing opticians. Although very convenient and largely used by histologists, they are not absolutely necessary.

Effect of Heat.—Mount a little potato starch in water, cover with a coverslip, and then gently warm the slide by holding it over a very small gas or spirit flame, taking care to apply the heat just beyond the coverslip. Directly the opaque starch grains near the edge of the coverslip become suddenly translucent, withdraw the heat and cool the slide. Examine under the microscope, observing first the grains furthest removed from the heated end of the slide. These, or at least some of them, should be intact and show no difference from grains that have not been so treated. Proceed towards the heated end of the slide, and observe, in successive degrees, the effect of moist heat upon the grains. First the hilum fissures, and here disorganisation commences, extending, in grains that have been more affected, along the long axis of the grain, often forming a **V**-shaped fissure. The central portion becomes first

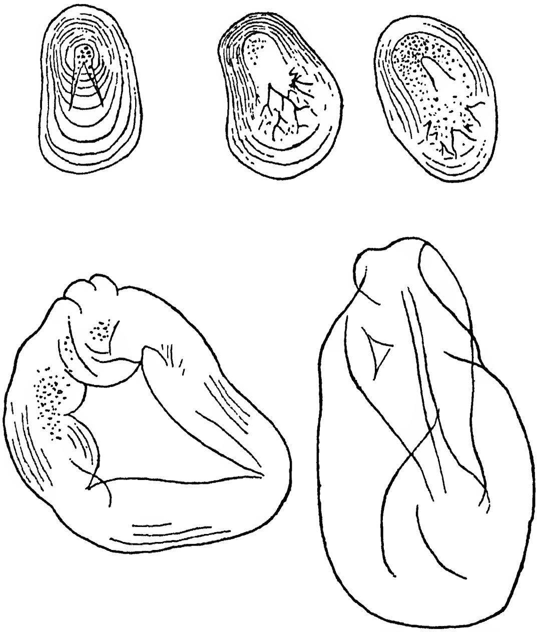

FIG. 2.—Potato Starch, showing various stages in gelatinisation.

granular, then translucent, and finally the whole grain swells considerably and is converted into a gelatinous mass in which only delicate dark lines are visible (fig. 2).

All starches gelatinise when heated with water, but the temperature at which gelatinisation is effected varies considerably with the variety of starch. The following table shows the temperatures that have been determined for some of the more important starches:

—	Distinct swelling	Begins to gelatinise	Complete gelat.
Rye	45·0	50·0	55·0
Rice	53·7	58·7	61·2
Barley	37·5	57·5	62·5
Potato	46·2	58·7	62·5
Maize	50·0	55·0	62·5
Wheat	50·0	65·0	67·5
Maranta	66·2	66·2	70·0
Acorn	57·5	77·5	87·5

The temperature at which gelatinisation takes place might, therefore, well be used to distinguish, for instance, between rye starch and wheat starch, and the attempt has even been made to base a quantitative separation upon this property.[1]

It is very important that the student should make himself acquainted with the effect of heat, and especially moist heat, upon starch grains. Many drugs, more particularly powdered drugs, are subjected to excessive heat during the process of preparing or drying, by which considerable alteration is effected in the appearance of the starch grains. These fissure, or become more or less translucent in the centre, or are even entirely gelatinised.[2]

Effect of Caustic Alkali.—Mount a little starch in water; place a drop of solution of caustic potash on the slide near the coverslip, and gently bring it into contact with the water in which the starch is mounted. If necessary, draw the alkali underneath the coverslip by applying a small fragment of filter paper to the opposite side. Observe the effect of the caustic alkali upon the grains. At first the striations become a little more distinct, then fainter; the central portion becomes transparent, as though solution were taking place; and finally the grain rapidly swells until the shape becomes unrecognisable.

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[1] Weinwurm, *Ztschr. f. Untersuchung d. Nahrungs- und Genussmittel*, 1898, p. 98.

[2] Compare figs. 15 and 17.

Solution of caustic potash is by no means the only reagent that will gelatinise starch. Concentrated solutions of chloral hydrate (five parts in two of water), of calcium chloride, of zinc chloride, &c., produce a similar effect. Solution of sodium salicylate (one part in eleven of water) gelatinises rye starch more readily than it does wheat or certain other starches, and hence has been utilised as a means of distinguishing them. Strong hydrochloric acid and strong sulphuric acid dissolve starch.

The presence of starch in a section often obscures important details and its removal becomes desirable. Solution of potash, or of chloral hydrate, or strong hydrochloric acid, will effect this, and such solutions are termed 'clearing agents.' Clearing may also be effected without the use of either acid or alkali by warming until the starch is gelatinised, and then digesting with diastase until solution is effected. Boiling with dilute mineral acid produces a similar result.

Iodine Test.—Mount a fresh slide in water and irrigate with iodine water (or solution of iodopotassium iodide diluted with water to the colour of dark sherry). Observe that the grains assume a pale violet-blue colour, which increases in intensity till it becomes almost black.

This is the most important chemical test for starch, and should be applied in all cases in which the identity of the particles under examination appears doubtful. For its successful application the presence of water is necessary, and it should be noted that, under certain circumstances, the blue colour may be overlooked. This is especially the case with very minute starch grains; these are best tested by a saturated solution of iodine in the solution of chloral hydrate previously mentioned. The chloral hydrate induces gelatinisation of the grain, while the iodine colours it blue, the blue compound thus produced being insoluble in the reagent used.

A few plants contain grains which resemble starch but fail to give the characteristic blue reaction with iodine, the colour produced being reddish or violet. To such grains the name of amylodextrin (see p. 22) has been given.

Polarisation.—Mount a little potato starch in water and examine it by polarised light. For the necessary appliances and the mode of using them reference should be made to one of the text-books of the microscope. With crossed prisms each starch grain shows a dark cross upon a light ground, the point of intersection of the arms of the cross being coincident with the hilum. This behaviour of starch is to be referred to its microcrystalline structure, and is occasionally of use in detecting starch grains that might otherwise be overlooked. It must be observed, however, that other vegetable substances (the walls of the cells, sphærocrystals of hesperidin, &c.) also rotate the ray of polarised light and may exhibit a similar dark cross upon a bright ground.

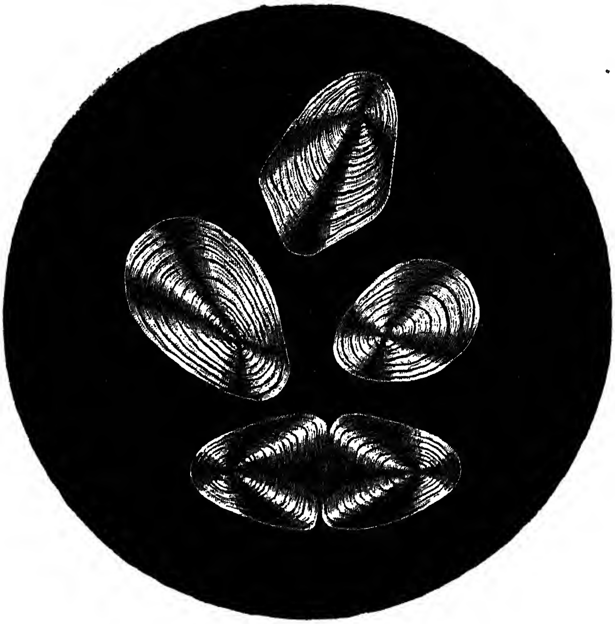

FIG. 3.—Potato Starch, in polarised light. (Behrens.)

Examination in Glycerin, &c.—Mount a little starch in pure glycerin; observe that the grains appear brighter and the striations become almost invisible, while the hilum is often conspicuous as a dark spot owing to a little air being imprisoned in the hollow centre.

Repeat the experiment with oil of cloves; the details are still less visible.

These experiments serve to show that glycerin and oil of cloves are unsuitable media in which to examine starch grains. The explanation is to be found in the fact that these liquids refract light more strongly than water does, and nearly as powerfully as the starch grain itself. Were the refractive power of the mounting medium the same as that of the grain, the latter would be invisible. The same applies to cell walls and fragments of cells ; these should be examined in water for the study of minute details, as the latter become almost invisible in strongly refractive media, just as the striations of starch grains disappear in oil of cloves.

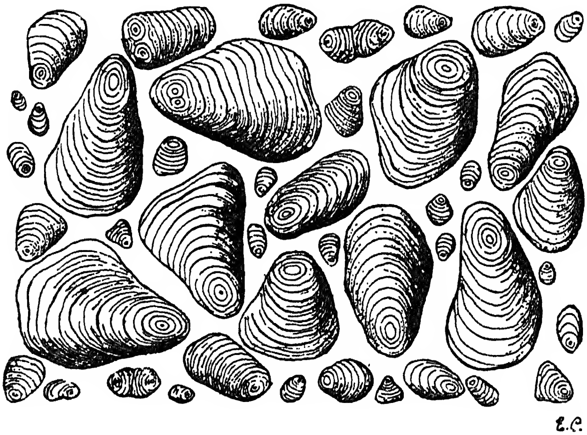

FIG. 4.—Potato Starch. ×240. (Greenish and Collin.)

Important Starches and their Characters

(1) **Potato Starch.**—Potato starch (fig. 4) is obtained from the tubers of *Solanum tuberosum*, Linn. It is composed of grains of variable size, some being so large as to be visible to the naked eye. Typical grains of this starch are flattened, and have an oval, ovate, ellipsoidal, or conchoidal outline. The hilum is punctiform and eccentric, being generally situated near the narrow end of the grain ; it is surrounded by numerous distinct concentric striations, some few of which are much more conspicuous than the others. In addition to these typical grains there are a few others, smaller in size and rounded in outline, or rounded on one side and flattened on the other, the last

named being sometimes attached by their flat sides in twos or threes.

The largest grains vary in length from 75 to 110 μ, those of medium size from 45 to 65 μ, and the smallest ones from 15 to 25 μ.

(2) **Maranta Starch.**—Maranta starch (fig. 5) is obtained from the rhizomes of *Maranta arundinacea*, Linn., and other species of *Maranta*. It is commonly known in commerce as ' arrowroot '—a term, however, which is also applied to the starches of other and widely different plants.

The different varieties of arrowroot are distinguished in trade by their geographical sources. Maranta starch is known as Bermuda, St. Vincent, West Indian, or Natal arrowroot, according to the country in which it is prepared.

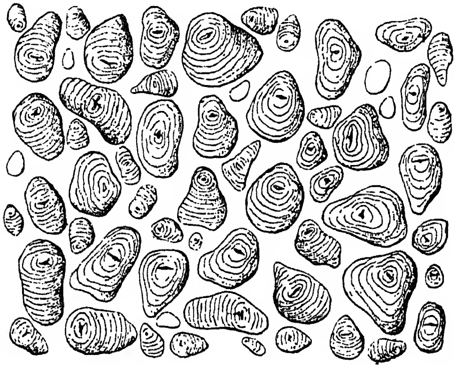

FIG. 5.—Maranta Starch. ×240. (Greenish and Collin.)

The grains of Maranta starch are simple and rather large. They are irregular in shape, the smallest being nearly spherical, while the larger are rounded, ovoid, pear-shaped, or sometimes almost triangular. The largest bear numerous fine concentric striations, and a conspicuous rounded, linear, or stellate eccentric hilum. In some varieties of arrowroot (Natal) the rounded hilum predominates, in others (St. Vincent) the linear or stellate, the latter often resembling the wings of a poised bird. The grains average about 30 to 40 μ in length, but may attain to 45, 60, or even 75 μ, as, for instance, in Bermuda arrowroot; the smaller grains vary from 7 to 15 μ.

(3) **Maize Starch.**—The starch obtained from the fruits of *Zea Mays*, Linn.

Take a grain of maize and cut it longitudinally into two parts. The centre of the grain is white and mealy; on one side of the whitish part, or partially surrounding it, is a yellowish horny portion, while on the other side is a greyish portion in which the embryo and scutellum can be discerned; the whitish mealy and yellowish horny portions constitute the endosperm. The reserve starch with which the endosperm is filled forms the maize starch of commerce.

Remove a little of the starchy central portion and examine it in water.

The starch grains appear rounded or muller-shaped; a few are polygonal. They are simple, and vary in size from 5 to 20 μ, but are on the whole tolerably uniform, the majority measuring from 12 to 18 μ. The hilum is mostly distinct, sometimes as a point, but more often as a two-, three-, or four-armed cleft; striations are not discernible.

Cut off from another grain a little of the horny portion of the endosperm and soak it in water for twenty-four to forty-eight hours. It will soften. Remove a small portion about the size of a pin's head and break it up with the dissecting needles in a drop of water. Examine it.

The starch grains are partly free, partly still contained, closely packed, in the cells of the endosperm.

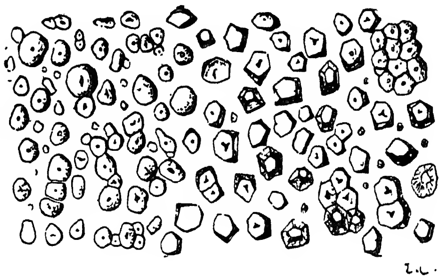

FIG. 6.—Maize Starch. ×240. (Greenish and Collin.)

The free grains are, like those in the previous preparation, simple and tolerably uniform in size, but they differ in being mostly polygonal with rather rounded angles. In outline they are often pentagonal. The hilum is frequently a point, and often exhibits two, three, or four radiating clefts. Some grains are much fissured at the periphery. In others there appears to be a large central cavity or possibly gelatinous portion surrounded by a brighter peripheral layer, the latter being often radially striated or fissured. These grains are usually larger than the others, and appear to have undergone partial gelatinisation. In none of the grains can concentric striation be detected.

All these grains may be found in commercial maize starch, but those with large central cavity are few in number.

(4) **Rice Starch.**—Rice starch is obtained from the fruits of *Oryza sativa*, Linn.

Soak a few grains of rice in water for three or four hours, then scrape off a little of the softened grain and mount it in water.

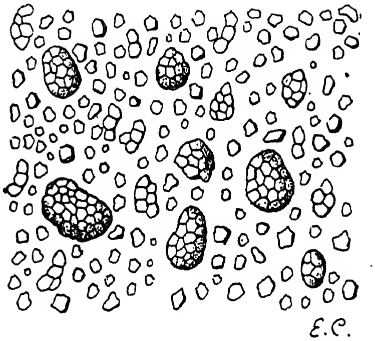

FIG. 7.—Rice Starch. ×240. (Greenish and Collin.)

The starch grains are very small and angular; their sides are mostly flat, but occasionally they are curved. There is seldom any hilum distinctly visible, but in some grains a central portion appears brighter—a difference possibly due to the drying of the grain. They are regular in appearance and uniform in size, averaging about 6 μ in diameter, but grains up to 8 or even 10 μ may be found, as well as some that are very minute.

Among these angular grains there will be found larger masses consisting of a number of grains compacted together (compound grains); they are ovoid or nearly spherical in shape, and measure from 20 to 30 μ in length. By pressure they easily break up into their component grains, and hence they are seldom found intact in commercial rice starch.

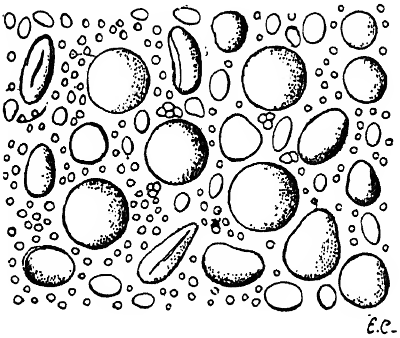

FIG. 8.—Wheat Starch. ×240. (Greenish and Collin.)

(5) **Wheat Starch.**—Wheat starch is obtained from the fruits of several species of *Triticum*.

Soak a few grains of wheat in water for twenty-four hours. Cut one transversely, and mount a little of the starch in water.

Examine it with the low power; observe that it consists of large rounded grains mixed with numerous small ones; grains of intermediate size are comparatively rare.

Examine with a high power. The large grains appear rounded, or nearly oval, without evident hilum or striations, but on careful examination one may be found here and there with a distinct hilum in the shape of a point, or cleft, or apparent cavity, and an occasional grain will also exhibit delicate concentric striation. In some samples of wheat most of the grains may show distinct striation.

Make the grains roll by moving the coverslip, or by pressing on one side of it. As the large grains roll they will appear oval or concavo-convex in outline, showing that the grains are not spherical but lenticular or bun-shaped; some will exhibit a darker longitudinal line in the centre, corresponding to the central cavity previously mentioned.

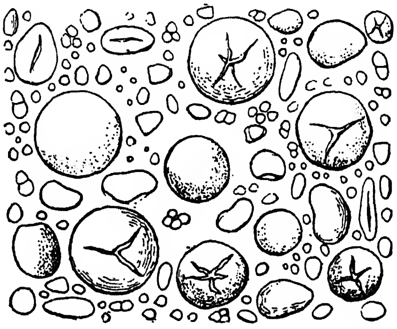
FIG. 9.—Rye Starch. ×240. (Greenish and Collin.)

In diameter the large grains measure mostly from 20 to 35 μ (when lying flat).

(6) **Rye Starch.**—Rye starch may be obtained from the fruits of *Secale cereale*, Linn. Rye starch closely resembles wheat, but the large grains attain a larger size (45 to 50 μ), and many of them exhibit a dark central cavity with several radiating clefts. Most of the larger grains are gelatinised when mounted in a solution of sodium salicylate (1 part in 11 parts of water) and kept for 24 hours; wheat and other starches are but little affected.

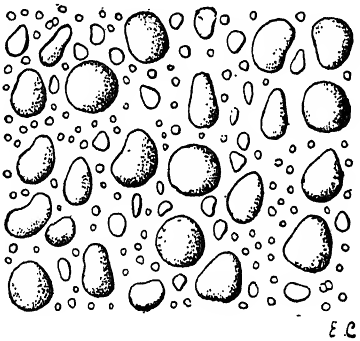
FIG. 10.—Barley Starch. ×240. (Greenish and Collin.)

(7) **Barley Starch.**—Barley starch is contained in the fruits of *Hordeum distichon*, Linn. Barley starch also closely resembles wheat, but the large grains are rather smaller, the majority measuring 18 to 25 μ, a few as much as 30 μ. They

are also less regularly circular, showing a tendency to bulge on one side and thus assume a sub-reniform shape (compare pea starch). In side view they are elliptical or lemon-shaped rather than lenticular.

Note.—Wheat starch is a common article of commerce, but rye and barley starches are not. The detection of barley starch when mixed with wheat starch is difficult, but the identification of the corresponding flours is easier, for these always contain fragments of the pericarp, &c., which offer additional and very valuable evidence of identity (compare 'Wheat,' in Section XI, p. 299).

(8) **Oat Starch.** — Oat starch is contained in the fruits of *Avena sativa,* Linn. It is not met with as an article of commerce, but an examination of the starch is desirable, as oatmeal is used for a variety of purposes.

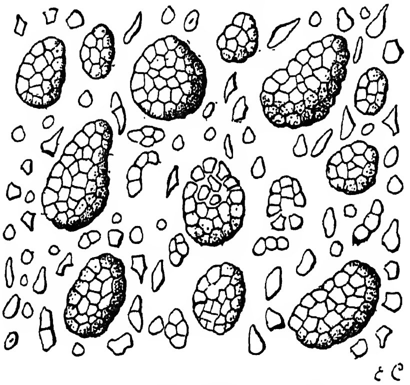

FIG. 11.—Oat Starch. ×240. (Greenish and Collin.)

When examined under the low power it appears to consist of a mixture of large and small grains, but, when the former are examined under the high power, they are seen to be compound grains consisting of a large number of small angular grains, into which they readily separate. In this respect the starch resembles rice starch.

Most of the component grains are angular, but some are lemon-shaped, round or semicircular; these form a valuable means of distinguishing the starch from rice starch. Neither hilum nor striations are visible.

The simple grains average about 10 μ in diameter and the compound 35 to 45 μ.

Oatmeal contains fragments of the seed-coats, pericarp, and paleæ, which may be utilised to establish the identity of the meal.

(9) **Bean Starch.**—(Fig. 128, p. 238) Prepare a little starch from a haricot bean (*Phaseolus vulgaris,* Linn.) in the same manner that maize and rice starch were prepared.

The larger grains are ovoid, elliptical, or somewhat reniform in outline; sometimes they are obscurely three- or four-sided, with very rounded angles, the smaller grains being often rounded. Through the hilum there usually extends along the long axis of the grain, and almost from end to end of it, a large, irregular, branching cleft that appears nearly black and is therefore very conspicuous; more delicate fissures often radiate towards the periphery. The striations are usually well marked. In length the grains vary from 25 to 60 μ, 30 to 35 μ being common measurements.

Examined in glycerin or in alcohol, the grains exhibit no cleft, but this gradually appears when they are irrigated with water. This phenomenon is probably due to swelling of the grain in contact with water.

The starches of the commoner leguminous foods (pea, bean, lentil, &c.) exhibit a remarkable similarity in size and shape. It is not, therefore, very difficult to recognise such a starch as being derived from a leguminous plant, but the determination of the species that has yielded it, is by no means easy (compare the descriptions and illustrations of leguminous flours in Section X).

(10) **Pea Starch.**—(Fig. 125) The starch of the seeds of *Pisum sativum*, Linn. The grains of pea starch are rather smaller than those of bean starch, the majority measuring from 20 to 40 μ. Some of the grains are oval-oblong, others rounded, or sub-reniform in shape; many are irregularly enlarged, so that their outline appears composed of arcs of circles of varying radius. The appearance of the hilum varies. In some specimens there is a conspicuous dark central cleft, as in bean starch; usually, however, it is less branched, smaller, and less conspicuous. In others there are but few grains with such clefts, the majority possessing simply an elongated hilum. Dark transverse clefts are also occasionally to be found. Radial fissures are comparatively common. Most grains are also distinctly striated, especially near the periphery, the central portion often presenting a more or less homogeneous appearance.

(11) **Lentil Starch.**—(Fig. 127) The starch of the seeds of *Lens esculenta*, Moench.

Lentil starch is intermediate in character between pea starch and bean starch. Most of the grains are simple and elliptical, ovoid, or rounded in shape; some of them exhibit irregular enlargements, like those of the typical grain of pea starch. The hilum is less branched and less conspicuous than that of bean starch, and usually not so dark; it often appears as a dark cleft extending almost the entire length of an oval grain, which then bears some resemblance to the flat side of a coffee bean. The striations are delicate, but they are usually visible. Most of the grains measure from 20 to 40 μ in length.

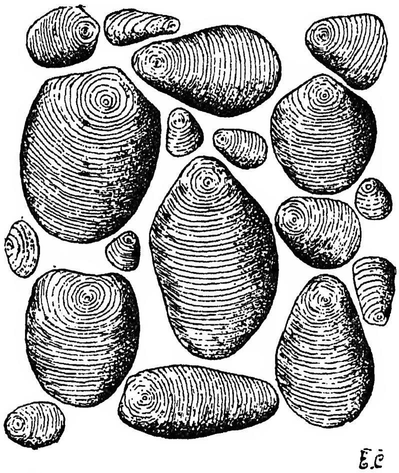

FIG. 12.—Tous-les-mois Starch. ×240. (Greenish and Collin.)

(12) **Tous-les-mois.**—The starch known as Tous-les-mois or Queensland arrowroot is obtained from the rhizomes of *Canna edulis*, Linn., and other species of *Canna*. The grains recall those of potato starch, but they are much larger, most of them ranging from 60 to 95 μ, although they occasionally reach 130 μ.

In shape they are broadly ovoid, oblong, or elliptical; many are flattened at the anterior extremity, and bear in addition a slight prominence opposite the hilum. In some grains similar prominences are observable on the sides, in others there is a distinct shallow depression on one side. The hilum is usually a point, often dark and, in most grains, very eccentric ($\frac{1}{5}$ to $\frac{1}{7}$). The striations are very distinct and regular. The grains differ, therefore, from potato starch in size, shape, and striation.

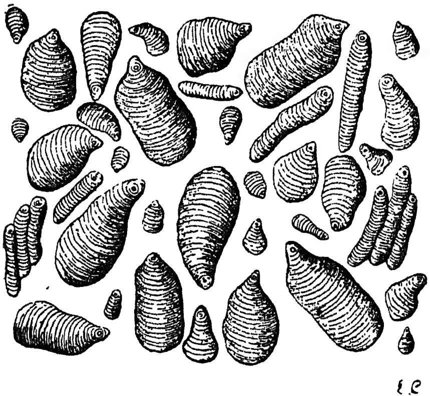

FIG. 13.—Curcuma Starch. ×240. (Greenish and Collin.)

(13) **Curcuma Starch.**—The starch obtained from *Curcuma angustifolia*, Roxb., and other species of *Curcuma.* It is often termed East Indian arrowroot. The grains are broadly ovoid or oblong, sometimes inclining to reniform or sub-reniform; they are usually rounded at the posterior extremity, but often taper rather abruptly at the other, frequently terminating in a nipple-like projection, in or near which the hilum is situated. The latter is commonly very eccentric; when not visible its position can be ascertained by following the concentric striations, which are usually distinct, at least in the large grains. The grains are so flat that when viewed on their edges they appear rod-shaped. The average size is from 36 to 60 μ.

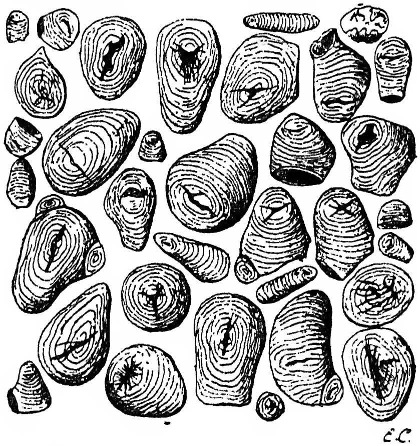

Fig. 14.—Sago Starch. × 240. (Greenish and Collin.)

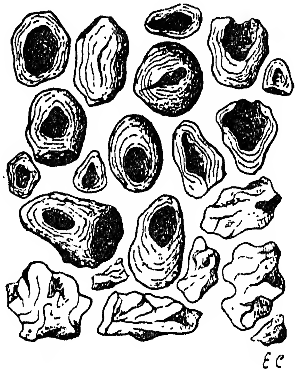

Fig. 15.—Sago. × 240. (Greenish and Collin.)

Many scitaminaceous plants contain a starch of this type.

(14) **Ginger Starch** (Fig. 168).—The starch of *Zingiber officinale*, Roscoe. Ginger starch resembles curcuma starch in general aspect. The grains are, however, smaller, varying mostly from 20 to 35 μ in length; they are rather thicker, and bear some resemblance to a sack tied at the neck.

(15) **Sago.**—Sago is prepared from the moist starch of the sago palm, *Metroxylon Sagu*, Rottb., by heating and stirring it until it agglomerates into small rounded granules; this form of the starch is often distinguished as pearl sago.

Soak a little sago in water for a few hours; crush a granule or two on the slide with water, and examine.

Some of the starch grains have been more or less gelatinised by moisture and heat.

Observe, first, the starch grains that are intact or nearly so. They are ovoid, broadly ovoid, muller-shaped, or irregularly three- or four-sided with rounded angles. Some are simple, but others bear, on as many short protuberances, one, two, or three flattened surfaces to which smaller grains have been attached, thus forming compound grains. Small grains may occasionally be found presenting one flat surface. The hilum is eccentric, but is usually more or less altered by gelatinisation. In most of the grains the striations are only indicated, but in some they are very distinct. In length the intact grains vary from 30 to 50 or 60 μ.

Next proceed to study the grains that have been altered to varying degrees by the moist heat to which they have been subjected. In many of these gelatinisation has commenced near the hilum, and converted the central portion into a transparent gelatinous mass ; in such grains fissures may be seen leading towards the posterior extremity. In others a considerable proportion (one-fourth or one-third) of the grain has been gelatinised; others have been converted into a gelatinous mass in which but little structure is visible, although the original shape may be to some extent retained; these have been usually enlarged by swelling. Lastly, numerous *débris* of completely gelatinised grains may be found (compare the experiment on the gelatinisation of potato starch).

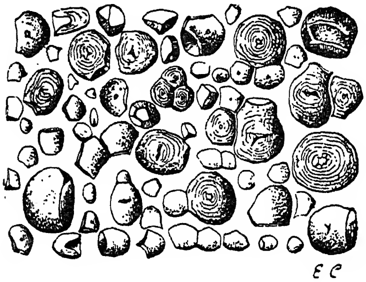

FIG. 16.—Tapioca Starch. x 240. (Greenish and Collin.)

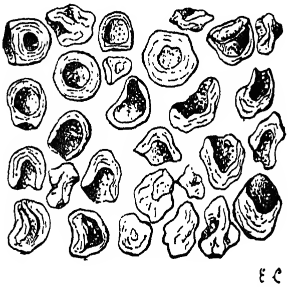

FIG. 17.—Tapioca. x 240. (Greenish and Collin.)

(16) **Tapioca.**—Tapioca is prepared from the starch obtained from the tubers of *Manihot utilissima*, Pohl. The method of preparation resembles that of sago.

Treat tapioca as directed for sago.

Examine the intact starch grains first; they are small, and the presence of one or two flat surfaces points to their having formed component parts of compound grains. Many are muller-shaped; they often have one flat surface, sometimes two forming an angle where they meet. The hilum is usually a point or small cleft, and eccentric. The grains that appear muller-shaped when lying on their sides are circular when standing on end, and then the hilum appears central. Striations are visible only in some of the larger grains.

The majority of the ungelatinised grains vary from 15 to 25 μ in length, but they may reach as much as 36 μ, those that are gelatinised being much larger.

Many of the grains exhibit the effects of heat similar to those shown by sago. The centre first becomes gelatinous, then radial fissures arise, especially in the peripheral portion. In many grains that are completely swollen the original shape of the grain is distinguishable; others have become converted into a shapeless gelatinous mass.

Dextrin.—Crude dextrin can be conveniently examined in alcohol, in which it is insoluble, and which effects no appreciable alteration in it. The starch grains from which it has been prepared have usually suffered but little visible change, and the variety can therefore be easily identified; usually it is potato starch.

On irrigating with water the grains swell a little and become translucent, the change progressing from the periphery towards the centre; the striations become very conspicuous, the grains partially dissolve, and finally solution becomes almost complete.

Dextrins that have been prepared by solution in water, decolorisation, and evaporation exhibit no such structure, the particles being amorphous, colourless, and angular; they assume a reddish colour with solution of iodine.

Amylodextrin.—Here and there in the vegetable kingdom, among drugs and spices, particularly in mace, parenchymatous cells are to be observed which contain small irregular granules. These granules vary exceedingly in shape. They are often elongated, with irregular wavy outline, and not unfrequently exhibit rounded or oval protuberances. They are seldom oval or rounded, as starch grains are. In size they range from 5 to 15 μ. They are characterised also by yielding a reddish-brown or reddish-violet coloration with iodine, a reaction which distinguishes them at once from starch grains.

These remarkable granules are believed to consist of amylose, together with amylodextrin and a variety of dextrin that is not coloured by iodine. Amylodextrin is intermediate between starch and dextrin.

Notes on the Examination of Starch

The adulteration of one starch with another is detected by mounting a carefully bulked sample in water, examining with the microscope, and comparing, if necessary, with a sample of known genuineness. No report should be given without, if possible, such comparison.

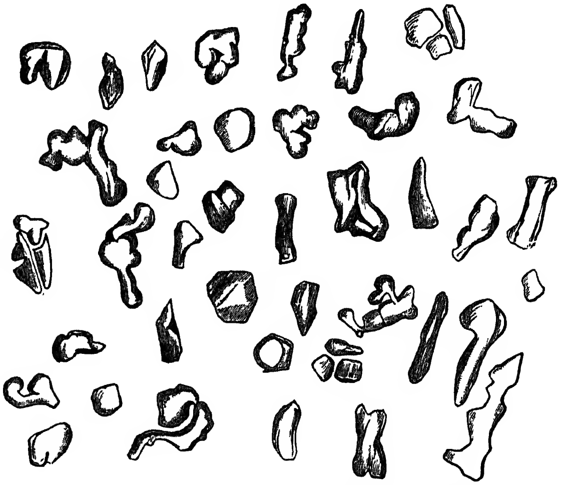

FIG. 18.—Amylodextrin of Mace. (Tschirch.)

The detection of starch as an adulterant of other (especially powdered) drugs should invariably be confirmed by the iodine test, in order to avoid mistaking minute particles of other substances (aleurone grains, fat, &c.) for starch grains.

In the examination of a starch for substances other than starch it is often advisable to remove by suitable means the starch that is present, and thus concentrate into a small compass any foreign substance present. This may be effected by either of the following methods :

(*a*) Mix 5 grammes of the starch with 10 grammes of 25 per cent. hydrochloric acid, warm to 40° C., dilute with 10 grammes of water, and allow the insoluble particles to subside.

(*b*) Boil 2 grammes of the starch with 20 c.c. water, cool to about 40° C., and add 10 c.c. of a filtered infusion of malt; keep the mixture at about 40° C. until the gelatinised starch is dissolved. It can then be set aside to deposit.

In both cases the use of a centrifuge will rapidly effect the separation of the deposit, which can thus be quickly washed and examined.

Compare also the isolation of the fragments of bran from wheat flour (Section XI, p. 299).

SECTION II

HAIRS AND TEXTILE FIBRES

INTRODUCTION

The substances that were the subject of investigation in Section I required but very little preparation to bring them into a fit state for microscopical examination, and their structure was so simple that this examination was very easy. With textile fibres the case is rather different. Not many of them are met with in such a condition that they can be mounted and examined without previous preparation, and often both careful and accurate observation is required before the minute differences that distinguish one fibre from another can be detected. Even to the experienced microscopist the identification of a textile fibre frequently presents considerable difficulty.

Cotton and flax fibres frequently find their way into microscopical preparations, and it is therefore desirable that the student should be able to recognise them. Hemp, jute, and Manila hemp are among the most valuable textile fibres. Wool is an example of a common animal fibre.

Fibres are identified by their morphological characters rather than by their chemical reactions. The apex, base, lumen, thickness of the cell wall, various markings, presence of a cuticle, &c., afford the most valuable information. The chemical properties often vary considerably, according to the treatment the fibres have undergone. Bleaching may completely remove the lignin from lignified tissues and effect other changes. In employing microchemical reagents, care must be taken to apply them always in exactly the same manner.

It is not intended that the student should do more now than make himself familiar with the general characters of the most important fibres and the methods adopted in examining them.

Those that desire to pursue the study of this important subject further must be referred to works[1] dealing specially with it.

Cotton Wool

Source.—Cotton wool consists of the hairs that cover the seeds of various species of *Gossypium.* These hairs are separated from the seeds by machinery, and, after passing through various processes, are spun into yarn.

Mounting.—Procure, if possible, a little raw cotton, or, failing that, a little ordinary, non-absorbent cotton wool. Moisten a few threads with a drop of alcohol on the slide, allow most of the alcohol to evaporate, add a drop of water, cover with a coverslip, and examine under the higher power.

Examination.—The hairs usually resemble more or less twisted ribbons, the edges of which are considerably thickened (fig. 19, *a*, *b*), but sometimes the cell wall is thick, the lumen narrow, and the hair but little twisted. The surface is not quite smooth, but appears delicately granular, or striated, the striations being frequently oblique; these markings are on the cuticle which covers the hairs. With a little searching an apex as well as base of a hair may be found and examined.

Cotton hairs attain a very considerable length, ranging from 10·3 mm. (Bengal cotton) to 40·5 mm. (sea island cotton). In transverse section they exhibit a flattened, reniform, dumb-bell-shaped, or irregular outline, and a narrow elongated cavity; they vary from 0·011 to 0·042 mm. in width (Wiesner), and differ from many other fibres in being always isolated and seldom exhibiting a circular or polygonal section (compare p. 30).

The student should sketch the middle portion and apex of a hair.

Reactions. Irrigate a few hairs (if possible, of raw cotton) mounted in water with freshly prepared cuoxam (see list of reagents); they rapidly swell and dissolve, with the exception of a very thin, delicate membrane, the cuticle, which will encircle the swollen hair at intervals and produce constrictions sharply alternating with large expansions.

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[1] Wiesner, *Rohstoffe des Pflanzenreiches*, Vienna, 1902, Parts 7 and 8; von Höhnel, *Die Mikroskopie der technisch-verwendeten Faserstoffe* (2nd edition); Herzberg, *Papierprufung*, Berlin, 1907; Cross and Bevan, *Text Book of Paper Making*, London, 1907; Matthews, *Textile Fibres*, New York, 1909.

This reaction depends on the presence of and thickness of the cuticle and succeeds better with Indian than with American cotton, and better with raw than with bleached and manufactured cotton, from which the cuticle may be entirely absent.

Mount a fresh preparation in water ; remove the water ; add one or two drops of solution of iodine in potassium iodide (a 1 per cent. aqueous solution of potassium iodide, saturated with iodine: von Höhnel, 1887) ; allow the reagent sufficient time to be absorbed by the cotton ; remove the excess by filter paper so that the cotton is nearly dry, then add sulphuric acid (concentrated sulphuric acid 3 volumes, water 1 volume, glycerin 2 volumes: von Höhnel), cover with a coverslip and examine. The hairs assume a reddish- or bluish-violet colour[1]

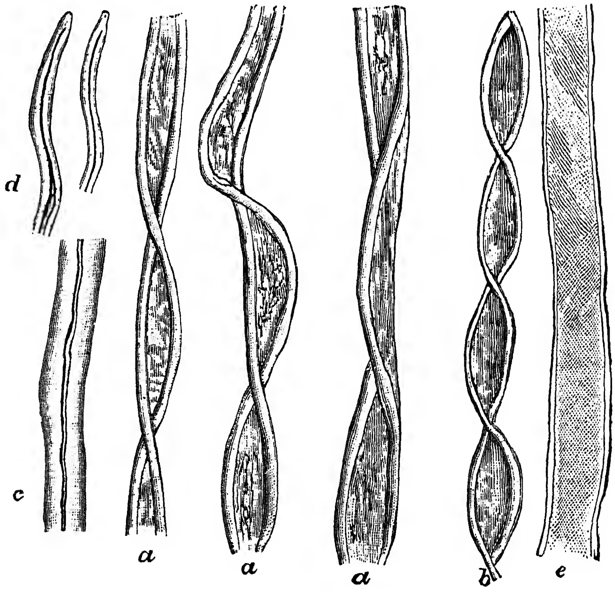

FIG. 19.—Cotton Fibres. *a*, *a*, *a*, central portions of mature hairs; *b*, weaker hair ; *c*, strongly thickened hair ; *d*, apices of hairs; *e*, dead hair from unripe seed. (Hanausek.)

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[1] Slight variation in the strength of the sulphuric acid reagent influences the colour produced ; the stronger the acid the more blue is the colour, but stronger acids are liable to produce swelling of the fibre, which should be avoided.

(cellulose reaction), whilst the dried protoplasm in the cavity remains brown.

Mount a fresh preparation in water; remove the water; dry by pressing on the fibres a piece of filter paper from which the loose fibres have first been removed by drawing it across the hand; drop on one drop of solution of chlorzinciodine, stir gently, cover and examine. In applying solution of chlorzinciodine to fibres do not deviate from this procedure, so that constant results may be ensured. The hairs gradually acquire a reddish- or bluish-violet coloration (cellulose reaction).

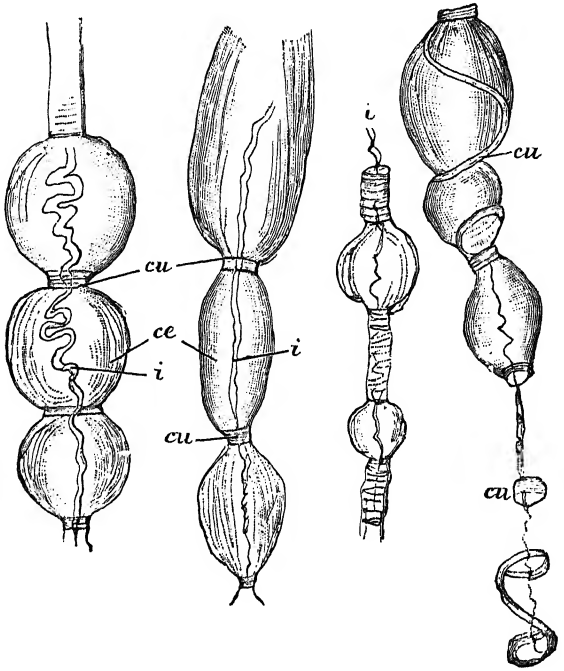

FIG. 20.—Cotton Fibres in cuoxam. *ce*, cellulose wall swollen by the reagent; *cu*, encircling rings of cuticle, which is not swollen; *i*, inner membrane lining the cavity. (Hanausek.)

Mount a few threads in a saturated aqueous solution of picric acid, warm gently for a few moments, and cool; the hairs are not stained yellow (distinction from animal wool, which stains yellow in hot solution of picric acid).

Diagnostic Characters.—Cotton fibres may be identified by the following characters :

(*a*) by the flattened, twisted fibre, with thickened edges;
(*b*) by the presence of an irregularly granular cuticle;
(*c*) by the blunt apex ;
(*d*) by the absence of transverse markings.

Flax

Source.—Flax consists of the bast fibres from the stem of *Linum usitatissimum*, Linn. These are separated from the plant by the processes of retting, scutching, heckling, &c., for a description of which reference should be made to one of the works dealing specially with the subject.

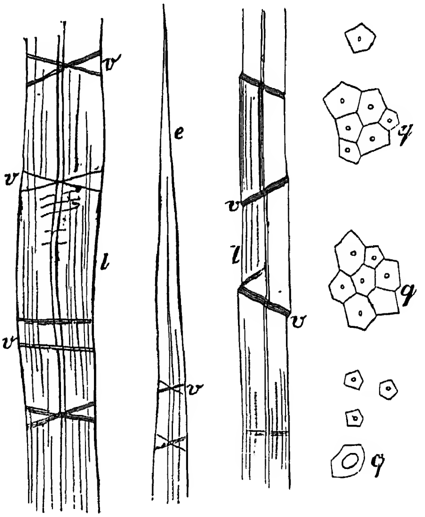

FIG. 21.—Flax Fibres. *e*, apex of a fibre; *l*, longitudinal aspect, × 400; *q*, transverse section, ×200; *v*, transverse markings. (von Höhnel.)

Mounting.— Procure from a rope-maker a little raw flax; cut off a few fragments, and examine them in water or chloral hydrate. The threads consist chiefly of *groups* of bast fibres. Here and there fragments of cell *débris* can be seen adhering to the fibres; these are the remains of the parenchyma of the flax stem, which has been imperfectly removed from the fibres. Particles also of cell contents of a greenish colour often remain attached to the fibres, and obscure their minute details.

Separation of the Fibres.—Boil a little flax in a 10 per cent. solution of sodium carbonate for half an hour, using a reflux condenser to prevent the solution from becoming too concentrated; pour off the alkaline solution, wash the flax with distilled water, and preserve it in dilute alcohol.

Take a little, and separate the constituent bast fibres by pulling them apart with the fingers, or by carefully teasing them out with the needles in a drop of water on the slide. Remove the water, mount in a drop of dilute glycerin, and examine.

Examination.—The individual fibres are now more or less completely separated from one another and freed from the cell *débris* and contents that were previously adhering to them. Under a high power the walls of each fibre are seen to be very thick, and faintly but distinctly striated; the cavity is narrow and uniform in width, and contains a little granular matter (remains of protoplasm).

Examine the walls minutely. Here and there delicate oblique lines can be detected, often crossing one another (fig. 21, *v*), and the fibres frequently show slight local enlargement. These appearances have been referred to injuries inflicted by the mechanical processes to which the fibres have been subjected; they may therefore vary in frequency in different varieties of flax. The uninjured bast fibres of the plant have been found to exhibit no such striations or protuberant swellings.

Mount a few prepared threads in chloral hydrate; the transverse lines are more distinct. Examine this preparation with the low power, and search for the tapering pointed ends of the fibres; a few of these are generally to be found.

Mount a few threads in chlorzinc iodine as directed on p. 27; the colour is darker than that given by cotton, and brownish rather than reddish-violet, the stronger fibres assuming a characteristic greyish colour.

Sketch a portion from the middle of a fibre, and also one of the ends.

Reactions.—The treatment with sodium carbonate does not appreciably interfere with the reactions of the fibre, of which the following are the most important:

Stain a few of the prepared and disintegrated fibres with iodine and sulphuric acid, as detailed for cotton. The fibres slowly assume a reddish- or bluish-violet colour; the transverse lines, being the first part to colour, are at that moment particularly conspicuous. The cavity contains a yellowish-brown granular substance (remains of protoplasm).

Mount a little of the flax in water and irrigate with cuoxam; the fibres swell and dissolve at once, leaving a minute thread from the centre undissolved. The swelling is often irregular and resembles that exhibited by many cotton fibres, but it does not show the sharp constrictions of the typical cotton fibre, nor are any delicate fragments of cuticle left after solution.

Preparation of Transverse Sections.[1]—Prepare transverse sections of some flax fibres as follows:

Take a number of threads of flax, without previous treatment with sodium carbonate, and draw them between the fingers until they are fairly straight; then moisten them with gum and glycerin and make them into a single rounded strand about as thick as a blanket-pin, taking care to keep the fibres straight. Let this strand dry thoroughly (in a warm place for twenty-four hours); fix it firmly in cork, and cut with a sharp razor transverse sections as thin as possible. Transfer these to a slide; drop on alcohol to free them from air, and mount in glycerin or a mixture of glycerin and alcohol; water may also be used, but many of the fibres, released from adhesion to the others, will assume a horizontal or oblique position.

Still better results may be obtained by first immersing the fibres in nitrobenzene for a few minutes, pressing between filter paper, rolling them in a solution of pyroxylin (5) and camphor (1) in ethyl acetate (94) and drying. The slender rod thus obtained may be repeatedly dipped in the pyroxylin solution, partially drying after each dipping, and finally dried by exposure to the air. The sections may be examined in water or dilute glycerin.

More elaborate methods of embedding in celloidin may be adopted, but the above process is simple and sufficient.

The section will exhibit a number of groups of bast fibres, each group consisting of several fibres. The fibres are very thick-walled and uniformly polygonal in outline, and have very small cavities. There are no intercellular spaces.

Diagnostic Characters.—The following are the chief diagnostic characters of flax fibres:

(*a*) The transverse sections are uniformly polygonal, and have very small rounded cavities; the fibres are usually grouped.

(*b*) The fibre consists entirely, or almost entirely, of cellulose; hence it yields a reddish- or bluish-violet colour with iodine and sulphuric acid and dissolves in cuoxam; there is no cuticle.

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[1] For the sake of completeness the directions for preparing transverse sections are introduced here, but the student who has not had considerable experience in section-cutting is recommended to content himself for the present with the separation and examination of the fibre.

Directions for cutting sections will be found under 'Ergot.'

(*c*) The fibres exhibit transverse lines.

(*d*) The ends taper gradually to fine points.

Hemp

Source. Hemp is obtained from *Cannabis sativa*, Linn. The fibres are separated from the stem by a method similar to that adopted for flax.

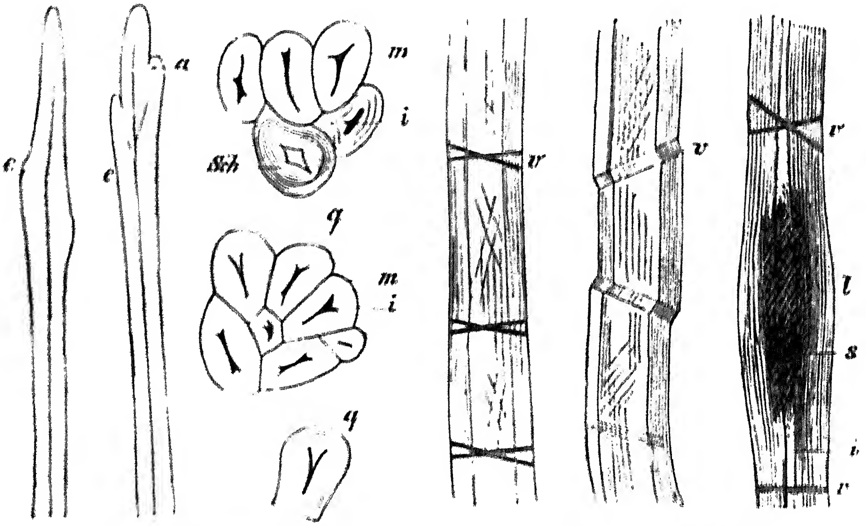

FIG. 22. Hemp Fibre. On the left, the apices of two fibres; on the right, portions from the middle; in the centre, transverse sections. *a*, short branch near the apex; *l*, lumen; *m*, middle lamella; *s*, *Sch*, striations; *v*, transverse or oblique markings. × 325. (von Höhnel.)

Preparation and Examination.—Prepare hemp for examination as directed for flax.

The fibres can also be separated by macerating in a 10 per cent. solution of chromic acid in dilute sulphuric acid until a strand taken out readily separates into its constituents; the remainder can then be washed, and examined as directed for flax.

Diagnostic Characters. Hemp fibres very closely resemble flax; they show similar swellings and transverse lines, and also consist almost entirely of cellulose. They may be distinguished by the following features:

(*a*) In transverse sections the cavities are comparatively large and oval or elongated.

(*b*) The ends of the fibres are usually blunt, or almost spathulate, and sometimes forked.

(*c*) They often have tufts of hairs at the knots.

(*d*) With chlorzinciodine they give a greenish colour, turning to reddish-violet.

(*e*) The coarser varieties often have cell *débris* attached to them, among which cells with brown contents, portions of the epidermis with warty hairs, and cells with rosettes of calcium oxalate are to be noted as distinctive.

Jute

Source.—Jute is obtained from *Corchorus olitorius*, Linn., and *C. capsularis*, Linn.

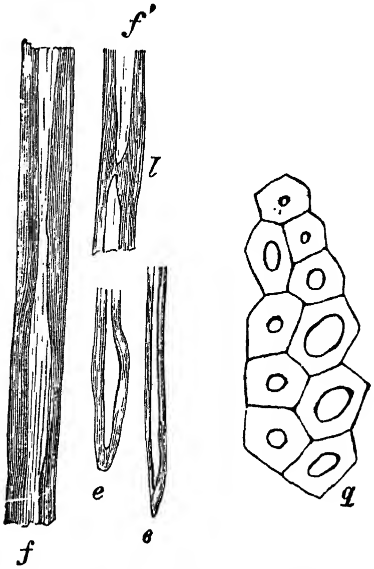

Fig. 23.—Jute. *e*, *e*, apices of fibres; *f*, fibre with constricted lumen; *f'*, fibre with septate cavity; *q*, transverse sections of fibres. (Hanausek.)

Preparation and Examination.—As directed for flax.

Diagnostic Characters.—

(*a*) Jute fibres are quite smooth; they show no longitudinal striations and no transverse lines.

(*b*) They are particularly distinguished by the size of the cavity, which is not uniform throughout the length of the fibre, but exhibits, at intervals, contractions caused by a corresponding increase in the thickness of the walls, which may occasionally be so great as to completely obliterate the cavity; this variation in the size of the cavity is conspicuous in the transverse sections of the fibres, some of which have large and others small cavities.

(*c*) They are lignified.[1]

(*d*) The ends of the fibres are blunt, sometimes almost spathulate.

Manila Hemp

Source.—Manila hemp is obtained from *Musa textilis*, Nées.

Preparation and Examination.—As for flax.

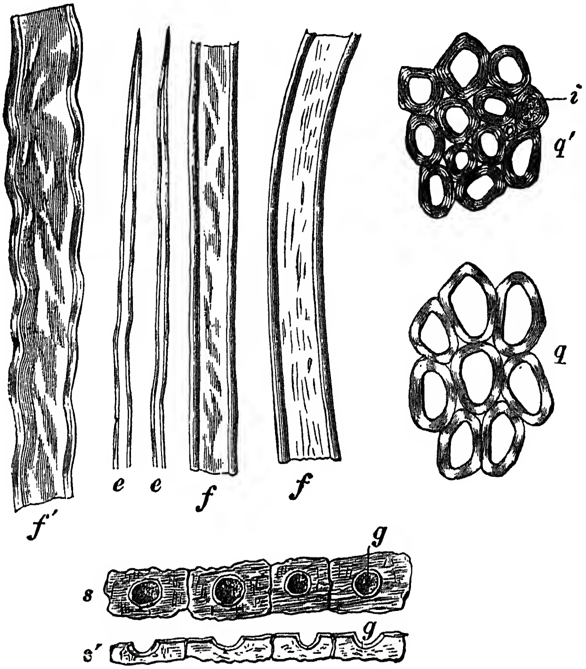

FIG. 24.—Manila Hemp. *e*, *e*, apices of fibres; *f*, *f*, central portions; *f'*, crushed fibre; *q*, transverse section of coarse fibres; *q'*, transverse section of fine fibres; *i*, fibre with proteid contents; *s*, *s'*, stegmata[2] with depressions, *g*. (Hanausek.)

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[1] Bleaching often destroys the lignin; bleached fibres may therefore give a bluish or violet colour with iodine and sulphuric acid, or with chlorzinciodine.

[2] Stegmata are cells that accompany the bast fibres of certain plants and remain attached to them when the fibres are separated from the stems. They occur only in ferns and monocotyledonous plants. Among the textile fibres, those derived from Musaceæ, Pandanaceæ, and Palmæ are alone characterised by the presence of stegmata. They generally contain nodules of silica, which may be found in the ash of the fibre, but the cell wall is not usually siliceous. (Wiesner.)

Diagnostic Characters.—

(*a*) The fibres are smooth, and show neither longitudinal nor transverse markings or swellings.

(*b*) The cavity is large and uniform, the fibres gradually tapering to fine pointed ends.

(*c*) The walls are lignified.

(*d*) The fibres are usually accompanied by oblong sclerenchymatous cells which are not stained by chlorzinc-iodine, but are coloured yellow to red by phloroglucin and hydrochloric acid.

(*e*) In section they are rounded or polygonal, and show large cavities and small intercellular spaces.

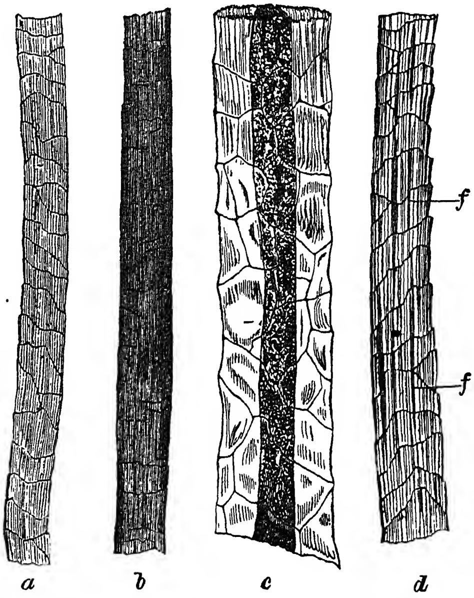

Fig. 25.—Various Forms of Fibre from Sheep's Wool. (Hanausek.)

Sheep's Wool

Source.—The raw, unwashed wool of the sheep.

Preparation.—If a few fibres of raw wool be examined in water, droplets of fatty matter will be seen adhering to them; these can be stained with tincture of alkanna (for details compare 'Lycopodium'), and consist of the wool fat naturally present in the fleece. It is desirable that this be removed before the wool is examined.

Warm a little raw wool gently in water, and pour off the cloudy liquid. Wash the residual wool with a little alcohol, and finally defat it with alcohol-ether, or with chloroform. Allow the defatted wool to dry.

Examination.—Mount a little in water. Each fibre exhibits an outer layer of overlapping, flattened cells with wavy walls; within these cells there is a fibrous tissue, and in the centre often a pith; the latter is not always visible.

Mount a little in cuoxam, warm gently, cool, and examine; the fibres stain bluish violet and the fibrous structure becomes very distinct; they do not swell or dissolve.

Mount another portion in solution of picric acid and warm; wash out with water; the fibres are stained yellow.

Stain another portion with iodine and sulphuric acid, as directed for cotton; the fibres stain yellow; they do not dissolve even when gently warmed.

Mount another portion in pure concentrated sulphuric acid and warm gently; they do not dissolve.

These reactions are amply sufficient to distinguish animal wool from the foregoing vegetable fibres. The identification of the source of the animal wool is, however, a matter of difficulty; in many cases it is at present impossible.

Silk

Source. True silk is the dry secretion of the larva of *Bombyx mori* (ordinary silk), *B. yamamaya* (Chinese silk), *B. cynthia* (Chinese, Japanese silk), *B. mylitta* (Tussore silk), and other species. The fibre consists normally of two threads which differ a little in appearance according as the fibre is derived from the outer, middle, or inner portion of the cocoon; that from the middle is the best and is used for spinning. Each fibre consists of an inner cylinder surrounded by a homogeneous, transversely interrupted layer of sericin of varying thickness. During the process of manufacture the sericin layer is more or less completely removed and the two constituent threads of each fibre are separated from one another.

Manufactured silk appears under the microscope as almost structureless, nearly cylindrical fibres to which here and there fragments of the sericin sheath may still be adhering. They are coloured brown by solution of iodopotassium iodide and are stained yellow by picric acid. They dissolve rapidly in concentrated sulphuric acid, and also in boiling concentrated hydrochloric acid, the silk of *B. mori* dissolving in half a minute, while the other varieties take longer. They are slowly soluble in cuoxam. These characters are sufficient to distinguish silk from the other fibres.

The student may advantageously supplement the examination of the foregoing fibres with the examination of a few samples of linen and cotton fabrics, lint, filter paper, &c., taking care to separate, in the fabrics, the warp from the weft, and examine each separately. In papers, especially in the finest qualities of filtering paper, the fibres often exhibit considerable disorganisation, due to the mechanical treatment to which they have been subjected, and are then difficult to recognise.

SECTION III

SPORES AND GLANDS

INTRODUCTION

The drugs that are treated of in this section do not require any elaborate preparation to fit them for microscopical observation. They differ from the substances that have already been discussed, inasmuch as their contents require particular examination.

Lycopodium

Source.—Lycopodium is a pale yellow, very mobile powder consisting of the spores of *Lycopodium clavatum*, Linn., and probably other species.

Mounting.—When thrown upon the surface of water lycopodium floats, and hence is with difficulty mounted in that medium for microscopical examination. The following method of procedure is the best: Place a small drop of alcohol on the slide, and mix a little lycopodium with it; allow most of the alcohol to evaporate, and then add a drop of dilute glycerin. The lycopodium will now mix easily with the dilute glycerin, and be free from the air bubbles which otherwise cling to it so pertinaciously.

Examination.—Examine first under the low and then under the high power. Observe the characteristic shape of the spore; it resembles a low, broad, triangular pyramid resting upon a convex base; this shape is evidently produced by the mutual pressure of the spores in the cell in which they were formed. Examine the delicate network that covers the convex base of each spore, and extends over the flat sides nearly, but not quite, to the angles they make with one another. This network consists of raised, colourless, transparent ridges, which appear as reticulate markings on the surface of the spore, but are seen on the edge to project above it. From the edge of the spore teeth appear to stand out, between which a delicate membrane is stretched; the teeth are the raised ridges seen edgewise, the membranes are the ridges seen lengthwise.

Sketching.—Proceed next to sketch three or four spores, selecting such as are lying in different positions, so that the sketch may represent different aspects of the spore. Draw the outline of the spore first. Next count the number of reticulations that are visible both transversely and longitudinally; introduce these into the sketch, taking care to represent their shape as accurately as possible and preferably by single, not double, lines. In counting the number of reticulations raise and lower the tube of the microscope, if necessary, so as to focus successively each part of the surface observed. On the curved base, for instance, the reticulations are not all upon the same plane, and therefore cannot all be distinctly seen at one and the same moment. They are nevertheless represented on the sketch as though that were the case.

Examination of Contents.—Mount a fresh slide, using water in the place of dilute glycerin. Press the coverslip firmly on to the slide with the handle of a pen or of a brush, so as to crush some of the spores. Examine the slide under the microscope. Some of the spores will have burst, usually along one of the angles, and discharged their liquid contents, which assume the form of globules. If the spores do not burst, repeat the pressure on the coverslip.

Identify these globules as fixed oil by the following characters and reactions:

(1) They appear as globules of *uniform* colour, bounded by a *narrow* dark line; there is no broad black border or bright centre (distinction from air bubbles).

(2) Irrigate the slide with tincture of alkanna diluted with an equal volume of water at the moment of using; the globules absorb the colouring matter and assume a reddish tint.

(3) Irrigate a fresh slide with a drop or two of solution of osmic acid; the globules rapidly assume a dark brown colour, the whole spore gradually colouring as the reagent penetrates into it and comes in contact with the oil that it contains.

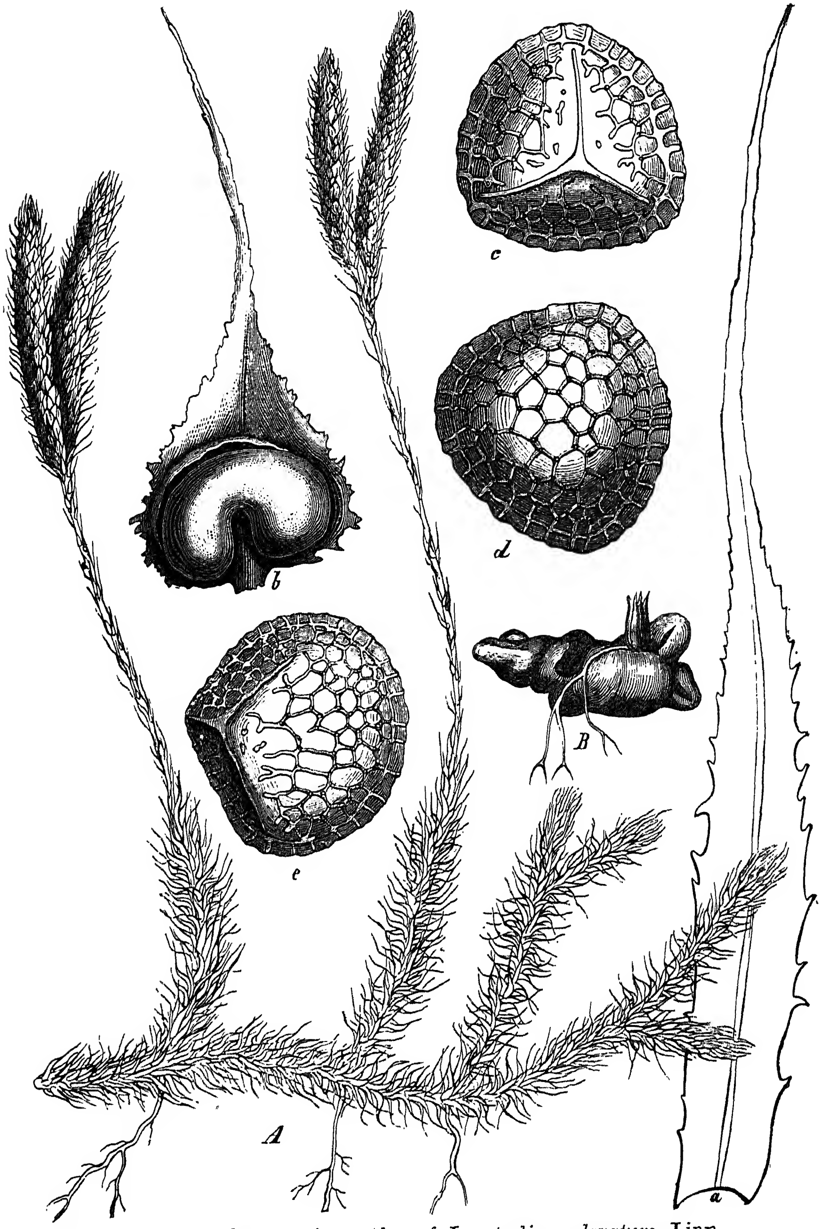

FIG. 26.—Lycopodium. *A*, portion of *Lycopodium clavatum*, Linn. (natural size); *a*, foliage leaf; *b*, sporophyll, with sporangium, magnified; *c*, *d*, *e*, spores magnified about 600 diameters; *B*, prothallium of an allied species. (Luerssen.)

(4) Irrigate a fresh slide with solution of Soudan red; the globules are coloured brilliant red.

The reactions 2, 3, and 4 are the principal colour reactions for fixed oil, and should be carefully studied. Volatile oils yield the same reactions, but may be distinguished by their ready solubility in alcohol.

Adulterations.—The most frequent adulterations of lycopodium are starch, the pollen of various plants, especially of coniferous trees (pine pollen), and powdered resin.

The pollen grains of most Abieteæ bear, on each side, a hollow vesicle produced by dilatation of the exine; hence their shape is extremely characteristic.

The pollen grains of the hazel are nearly spherical, and exhibit three pores. Those of most other plants differ so notably from lycopodium spores as to be distinguishable at first sight.

Starch may be recognised by its appearance and identified by the iodine test.

Resin readily soluble in alcohol is precipitated when the lycopodium, moistened with alcohol, is mixed with dilute glycerin; resin difficultly soluble in alcohol appears as colourless, angular fragments in the preparation.

Inorganic matter can also be detected under the microscope, but in this case chemical examination is to be preferred (determination of the ash, &c.)

Lupulin

Source.—Lupulin is a yellowish-brown granular powder consisting of the glands obtained from the strobiles of the hop, *Humulus Lupulus*, Linn.

Mounting.—Procure some fresh hops; from one of the strobiles remove some of the bracts; these may be recognised by the minute fruit enfolded at the base. Examine a bract with a hand lens, and observe the pale or golden yellow glands that are scattered over the base; this is lupulin.

Transfer some of the glands to a slide, using a needle or small brush for the purpose; or hold the bract over the slide and tap it smartly with the needle. Moisten with alcohol, and *immediately* add a drop of glycerin. Do not allow the alcohol to remain long in contact with the glands, as it rapidly penetrates them and dissolves the contents. Lower the coverslip carefully, for even slight pressure is sufficient to burst the glands and cause them to discharge their contents.

Examination.—Examine them under the low power. Each gland consists of a single hemispherical layer of cells, the common cuticle of which has been raised, dome-like, by the secretion of oil between it and the cell walls. This cuticle bears the impressions of the cell walls upon which it originally rested; they are frequently visible under the high power.

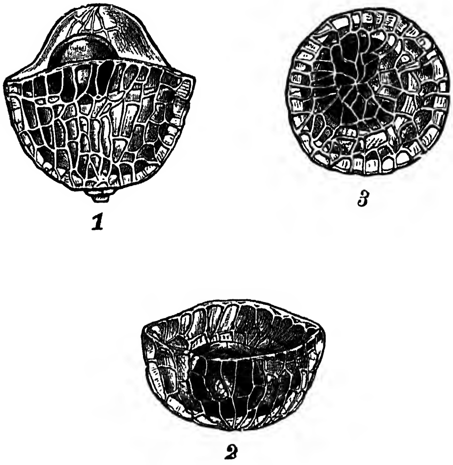

FIG. 27.—Lupulin Glands. ×100. 1 and 2, side views; 3, seen from below. (Vogl.)

The cells of which the lower half of the gland consists can frequently be distinctly discerned.

Sketching.—Sketch two or three glands in different positions, as described on p. 38.

Contents.—Crush the glands by pressing gently on the coverslip; observe the granular mixture of oil and protoplasm that exudes. The presence of oil can be detected by the reagents mentioned on p. 38.

Adulterations.—Lupulin is not often adulterated, but it is generally very impure, containing sand, vegetable *débris*, &c. These are easily distinguished from the characteristic glands. Sand is usually in colourless fragments of irregular angular shape. Vegetable *débris* can be recognised by their cellular structure (best seen after mounting in solution of chloral hydrate).

Note.—It is often necessary to prepare and examine several slides of lupulin before glands are found exhibiting their structure well. Too much pressure on the glands may be obviated by introducing a few splinters of a coverslip. Commercial lupulin is not well adapted for examination ; the glands are usually dark in colour and much shrunken, hence their structure is not easily discerned. The oil in the gland does not usually exist in the globular form shown in the figure, but will often assume it after treatment with solution of potash.

Kamala

Source.—Kamala is a fine, granular, mobile powder of a dull red colour. It consists of the hairs and glands that cover the fruits of *Mallotus philippinensis*, Müll. Arg. Even to the naked eye it appears heterogeneous.

Mounting.—Mount a little kamala as directed for lupulin. Examine with the low power ; groups of hairs and small garnet-red glands can be distinguished ; particles of sand and of vegetable tissue are often also present.

Examination.—Examine the preparation under the high power ; each group of hairs consists of a tuft of thick-walled, short, pointed, divergent hairs which contain sometimes air, sometimes a granular substance, sometimes a reddish resin. Occasionally they are divided by delicate transverse walls into two or more cells. Observe that the hairs are seldom single. The glands are so deep in colour that their structure cannot be discerned and it becomes necessary to subject them to some suitable treatment.

Mount a fresh portion of kamala in solution of potash without previously moistening it with alcohol ; the alkali readily dissolves the red resin, forming a deep brownish-red solution. Irrigate gently with solution of potash until the colour is almost completely removed.

Examine now with the low power, and, having selected a suitable gland, examine it more closely with the high power. It consists of a number of elongated cells, radiating in a more or less regular manner from a common centre, and enlarged at their free extremities. Observe that they are enclosed by a delicate membrane ; this is the cuticle that has originally covered the secreting cells, but has been raised in the same way as the cuticle of the hop gland. The resin has been contained in the space between the cuticle and the cells, secretion having taken place on the radial as well as the outer wall of each cell.

Sketching.—Sketch, under the high power, a small group of hairs and two or three glands in different positions. Represent accurately the apex, the thickness of the wall, and the shape of each hair.

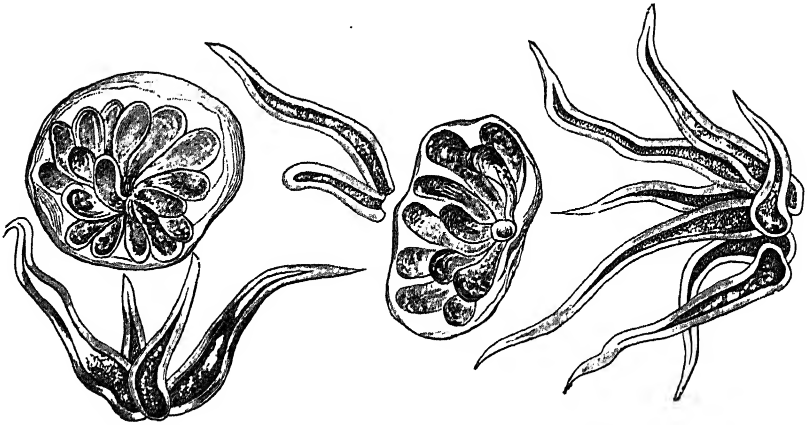

FIG. 28. Kamala (× about 140), showing two glands with their secreting cells and three groups of hairs. (Moeller.)

Adulterations.—Kamala is often grossly adulterated, but its characters are so well marked that it is easy to identify the hairs and glands of the genuine drug.

Vegetable *débris* often occur in it, but should not be present in excessive quantity. Minute fragments of reddish sand are frequently to be found in great numbers. Coloured starches have also been observed ; these can be identified in the usual way, after decolourising them, if necessary, with alcohol.

The hairs and glands of *Flemingia sp.* (wars or wurrus) bear a general resemblance to kamala, but the hairs are usually single, and the glands contain secreting cells arranged in four or five tiers, instead of radiately.

SECTION IV

ERGOT

Source.—Ergot is the compact mycelium of *Claviceps purpurea*, Tulasne, developed in the inflorescence of *Secale cereale*, Linn.

Preparation.—Put a few well-developed ergots into a dish or beaker with a little wet blotting paper; cover the dish, and allow them to remain for a few hours. After exposure in this way to an atmosphere loaded with moisture the ergots will lose their rigidity and become flexible. In this condition they are well suited for cutting.

Most drugs are either so hard or so brittle as to require softening before sections can be cut from them. The most favourable condition is an almost tough or waxy one; neither so hard as to afford great resistance to the razor, nor so soft as to be flaccid. In many cases this can be most satisfactorily attained by exposing the drug to a moist atmosphere until it has acquired the desired condition. Such treatment is well adapted for soft drugs, such as leaves generally (very leathery ones excepted), many parenchymatous roots and rhizomes, &c. It is often preferable to soaking in water, which is liable to make soft drugs too soft and flaccid, and entails free contact of the various cell contents with water, which is often undesirable. Hard drugs, on the other hand, such as woods, woody barks, many roots, rhizomes, &c., require soaking, often prolonged for several days, in water or dilute glycerin. Others, again, require hardening in alcohol after soaking in water, as, for instance, cloves. No specific instructions, therefore, applicable to all cases can be given, but the aim should be to bring the drug into a waxy condition, if possible without soaking it in any liquid at all.

Embedding.—One of the ergots properly softened must next be embedded in elder-pith ; this can usually be obtained from an optician or from one of the clockmakers in Clerkenwell. Select a piece about the thickness of the little finger and break off a couple of inches ; lay it on the table, and halve it lengthwise. Each half should have the shape of a half-cylinder. Cut in each half a depression to receive the ergot ; break the latter across the middle, and fix one half between the two pieces of elder-pith so that the transverse surface of the ergot is just below that of the elder-pith ; bind them firmly in this position with a strong thread. The ergot is now ready for cutting.

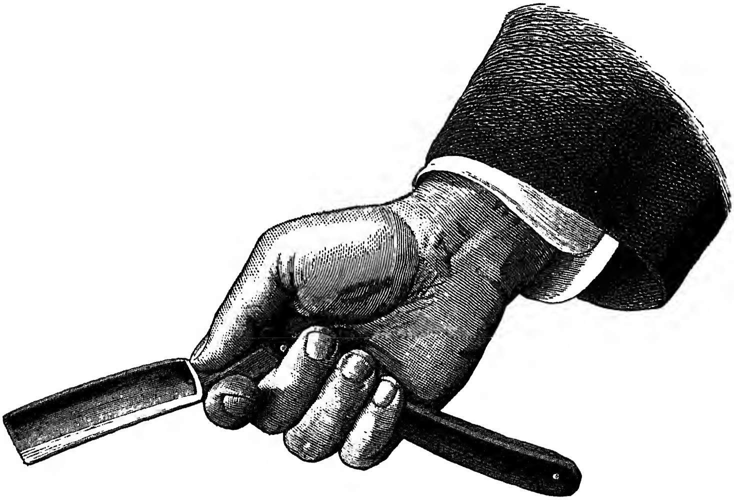

FIG. 29.—Showing the manner in which the razor should be grasped. (Behrens.)

Cutting Transverse Sections.—Bend the forefinger of the left hand and hold the elder-pith firmly but lightly between the thumb and the first knuckle-joint of the forefinger, the surface of the ergot being nearly level with the edge of the finger. Take the narrow steel tang of the razor between the thumb and forefinger of the right hand, so that the thumb is nearly parallel to the blade while the forefinger bends over it ; the remaining three fingers then bend over the handle of the razor, and thus hold it securely but lightly.

Holding now the elder-pith and razor as directed, rest the flat part of the blade on the forefinger of the left hand, with the heel of the blade near the pith; then draw the razor from left to right, gently advancing it towards and through the pith so as to cut off a thin slice. Use the edge of the forefinger as a guide for the razor blade, which should preferably have the side that rests upon the finger ground flat. Cut in this way about a dozen or more sections, transferring them from the blade of the razor to a small glass dish containing a little water.

Mounting and Examination.—Select one of the thinnest sections; transfer it with a small brush to a drop of water on a slide; cover with a coverslip and examine, first under the low, and then under the high power. Observe the margin of the ergot; it is a narrow dark brown line which on closer investigation, especially where the section is thin, is seen to be a layer of small cells with dark brownish contents. Here and there are fragments of ill-defined tissue outside the dark line; they are the remains of the outermost portion, which has perished. Within the brown line the cells show an oval, rounded, or elongated oval outline, the entire section consisting of such cells.

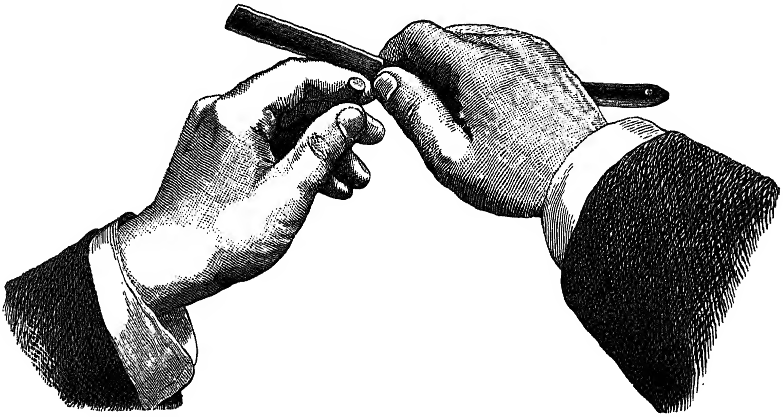

FIG. 30.—Showing the relative positions of the razor and the object in cutting sections by hand. (Behrens.)

Contents.—These cells contain minute globules, and similar globules may be found scattered in the mounting medium near the section. They resemble oil globules. Apply the osmic acid test; they gradually stain brown, showing that they consist, partly at least, of oil.

Mount a section in chloral hydrate; the oil collects into large globules. (The oil appears to exist intimately associated with proteid matter; this the chloral hydrate dissolves, setting free the oil in minute globules, which then easily unite into larger ones.) The cavities of the cells become more conspicuous.

Longitudinal Sections.—Cut now from an ergot, by two transverse cuts, a small piece about 3 millimetres long. Embed this in pith and cut longitudinal sections. Examine these. They present a structure very similar to that of the transverse section, but there are a greater number of cells with elongated oval outlines.

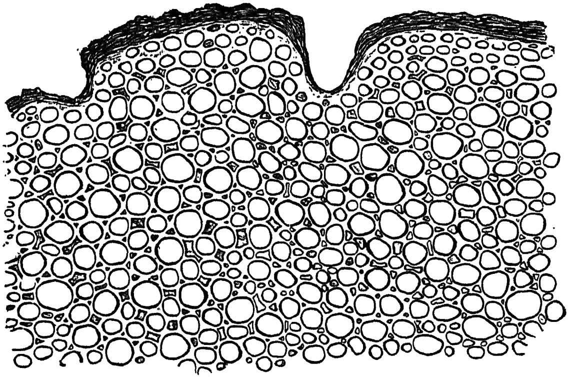

FIG. 31.—Ergot of Rye. Transverse section, cleared. (Vogl.)

The ergot consists of a number of tubular cells (hyphæ) which interlace with one another and are so compact as to form a solid body. Although the cells run in all directions, there is a somewhat greater tendency for them to assume a longitudinal than a transverse position; hence in the longitudinal section the cavities are slightly more elongated. These cells contain chiefly fixed oil (osmic acid reaction) and the remains of protoplasm.

SECTION V

WOODS

INTRODUCTION

Objects of the Investigation.—The student should now proceed to the examination of more complex organs. In approaching this part of the subject, he must bear in mind that the principal object sought is an accurate knowledge of the structure of the drug under examination. In the case of the woods, for example, his investigations should enable him to answer the following questions:

1. Of what elements is the wood under examination composed?
2. What is the exact nature of these elements, the form of the cells, the markings on the walls, the material of which they consist?
3. What are the contents of these elements (starch, calcium oxalate, resin, volatile oil, colouring matter, &c.)?
4. How are these elements arranged?
5. What are the particular characters of the elements present, or their arrangement, that would enable us to distinguish this drug from other similar ones?

From a medicinal point of view the woods can scarcely be called an important class of drugs, but the great variety of technical uses to which various woods have been put renders it very desirable for the student and analyst to be acquainted with the methods adopted in the examination of them, and with the chief features by which one wood may be distinguished from another. The question of the identity of a wood is constantly arising, the problem to be solved being, usually, whether the wood used for a particular purpose is the wood it purports to be, or whether a cheaper and less valuable one has been substituted for it. Wood pulp is very largely used in the manufacture of paper, and, consequently, in the examination of paper the recognition of wood pulp becomes very necessary, even after it has undergone the process of bleaching, by which the reactions yielded by the original wood are considerably modified. Wood may also be present in various surgical bandages, and is sometimes met with in powdered drugs, into which it may have been either intentionally or accidentally introduced.

Before proceeding to investigate the structure of medicinal woods, the student should study the general structure of wood in his text-book of botany.[1]

Definition.—By the term 'wood' botanists[2] understand all the secondary tissues produced by the cambium on its inner surface. This definition includes the softer, largely parenchymatous tissues that lie within the cambium ring of such a drug as calumba root, as well as the hard lignified tissues of which guaiacum, quassia wood, &c., are composed. In this section of the work, however, only those drugs that are grouped together by pharmacognosists under the term 'ligna' will be considered.

Typical Structural Elements.—The following are the chief types of elements that are met with in the woods, but it must not be forgotten that many intermediate forms occur.

1. Vessels.
2. Tracheids.
3. Wood fibres.
4. Wood parenchyma.
5. Cells of the secondary medullary rays.

1. *Vessels.*—These may usually be recognised by their comparatively large size, but they are more particularly characterised by the perforations in the transverse walls, by which a superposed row of cells has been converted into a vessel. The manipulations to which the tissues are subjected in separating the various elements from one another result in a separation of the vessel into the elements from which it was formed; these may be recognised by the perforations in the transverse walls (fig. 32, *a*).

The length of the vessels is not an important factor, but the width should be observed, although frequently vessels of

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[1] Vines, *Student's Text-Book of Botany*, p. 134 et seq., p. 196 et seq.

[2] Strasburger, Noll, Schenck, und Karsten, *Lehrbuch d. Botanik*, 1908, p. 130.

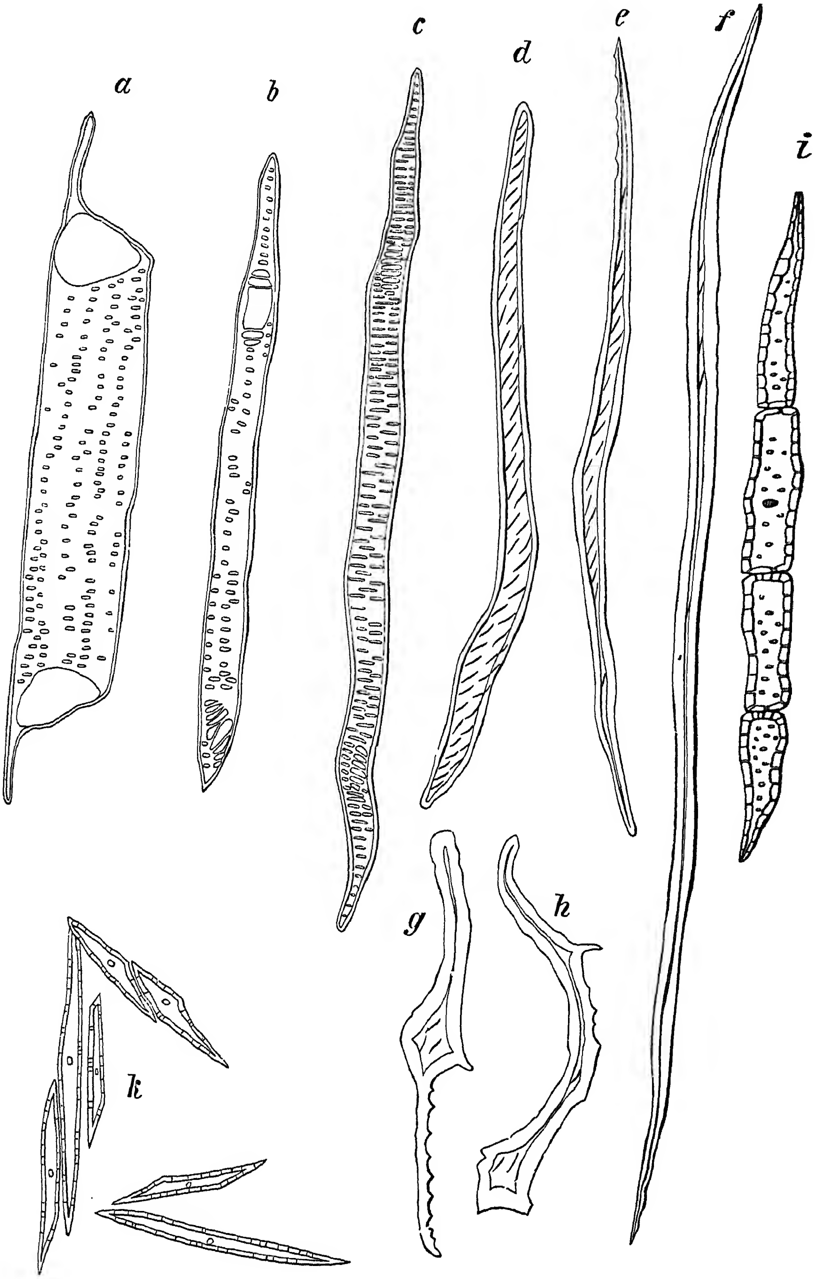

FIG. 32.—Elements of the Wood of the Copper Beech (*Fagus silvatica*). *a*, vessel; *b*, intermediate form; *c* and *d*, tracheids; *e* and *f*, wood fibres; *g* and *h*, wood fibres of irregular shape; *i*, wood parenchyma; *k*, cells of medullary ray. (Schwarz.)

varying size are found in the same drug. Indeed, the student should remember that absolute size is not always a very reliable character, as, under different circumstances, the absolute size may vary. Relative size, on the other hand, is more valuable, for the circumstances that increase the size of one element also increase that of the others, and the relative size therefore remains constant. More important than the size of the vessel are the size and character of the pits on its walls (fig. 33). These may be large or small, round, angular, or elongated; they may be simple or bordered; all such details should be carefully noted.

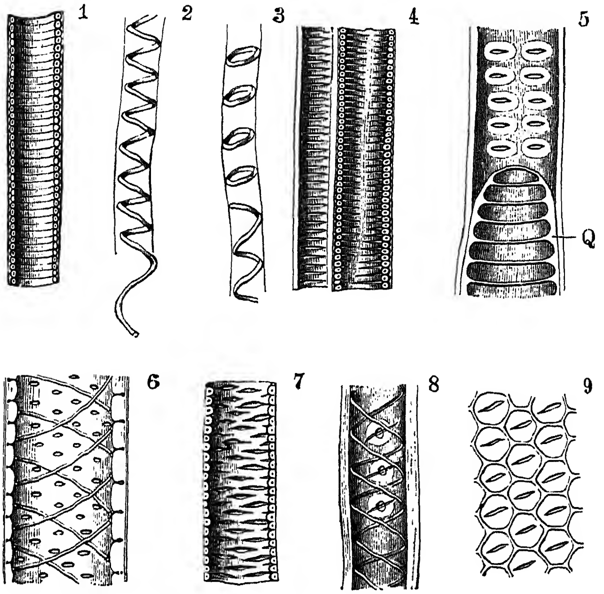

Fig. 33.—Various Forms of Vessels. 1 and 2, spiral; 3, annular, passing into spiral; 4, scalariform; 5, with bordered pits and scalariform perforation of oblique wall; 6, with simple pits and double spiral; 7, reticulated; 8, tracheid, with bordered pits and double spiral; 9, with bordered pits. (Vogl.)

The secondary wood never contains annular or spiral vessels.

2. *Tracheids.*—Tracheids are distinguished from vessels by their development from a single cambial cell, vessels being formed from a superposed row of cambial cells. They do not, therefore, as a rule, exhibit perforations by which superposed

tracheids communicate with one another (compare ipecacuanha wood, which is exceptional in this respect). They are usually elongated, and have rounded or tapering, but not sharply pointed, ends (fig. 32, *c*). Their chief function being the transmission of water, they are commonly free from cell contents, but in forms intermediate between tracheids and wood parenchyma they may serve as storage cells for reserve material. From true wood parenchyma they are distinguished especially by their prosenchymatous form; from vessels by their smaller size, and by the absence of perforations. Their walls usually bear small bordered pits, but in the tracheids of coniferous woods the pits are very large. In examining tracheids attention should be paid to the same points as mentioned in the previous paragraphs for vessels.

Cells that occupy a position intermediate between true tracheids and wood parenchyma have been called 'intermediate' or 'fibrous' cells. Like tracheids, each fibrous cell is developed from a single cambial cell, whereas several wood parenchyma cells are produced from a single cambial cell by transverse walls. They differ from true tracheids by being usually shorter and having more rounded ends, but particularly in serving as storage cells for reserve material. Fibrous cells may be thin-walled or thick-walled.

3. *Wood Fibres.*—These commonly form the principal constituent element of woods. They are usually long and narrow, and taper gradually at each end to a fine point (fig. 32, *e* and *f*). As their function is purely mechanical, they are destitute of protoplasmic contents, and do not act as storage cells for starch or other reserve material. They are developed from a single cambial cell, but their cavities are sometimes divided by very delicate transverse walls (chambered fibres). Their pits—which, as a rule, are not very numerous—are narrow, elongated slits, which may be simple or bordered, and are usually arranged in a left ascending spiral (left oblique). In determining the nature of the pits, care must be taken to examine longitudinal (radial or tangential) sections rather than the elements that have been separated from one another by digestion with nitric acid and potassium chlorate, or with chromic acid (see later), as after treatment with these reagents both the borders and the pits themselves are less easy to see. In determining the direction of the pits it is necessary to be quite sure

that the upper, and not the lower, wall of the cell is in focus, as one is very liable to focus through the cell to the lower wall. The breadth of the cell and the thickness of the wall should also be noted, as these details sometimes form means by which the wood fibres of one drug may be distinguished from those of another. The length may also be of service, but not for identifying powdered woods, as the fibres are in that case mostly broken. Although true wood fibres never contain starch, they may, nevertheless, contain resin (guaiacum wood, red sanders wood) or volatile oil (yellow sandal wood), &c. The colour of the fibres and the nature of their contents may therefore be valuable indications of identity.

4. *Wood Parenchyma.*—Typical wood parenchyma cells are developed from a cambial cell by transverse division, and this origin can often be traced in the parenchymatous cells isolated from woods ; very frequently a row of several superposed rectangular cells is terminated at both extremities by a bluntly conical one. Their walls are often moderately thick and freely pitted with simple pits. They often contain starch, calcium oxalate, &c.

The distribution of these cells, the thickness of their walls, the number, character, and distribution of the pits, as well as the nature of the contents, should be carefully determined.

5. *Cells of the Medullary Rays.*—When isolated these are scarcely to be distinguished from the cells of the wood parenchyma, but in sections, or in such fragments as are often found in powdered woods, they may be identified by their position and arrangement, for whereas wood parenchyma cells have their long axes parallel to the long axes of the wood fibres or vessels that accompany them, the long axes of the medullary ray cells are transverse to these.

Here, also, the same particulars should be studied as have been indicated for the wood parenchyma, and, in addition, the height and breadth of the medullary rays as exhibited by a tangential section.

Diagnostic Characters.—The principal diagnostic characters of woods are to be found in

(*a*) The elements of which the wood consists, especially the presence of any element of unusual form, or the absence of any element of general occurrence; for instance, the absence of wood fibres and wood parenchyma (pine wood, &c.), presence of oil cells, ducts, &c.

(*b*) The structural details of the elements present, especially the shape, thickness of wall, form and distribution of the pits.

(*c*) The distribution of the tissues, especially the arrangement of the wood parenchyma, grouping and distribution of the vessels, the width and height of the medullary rays.

(*d*) The contents of these cells, particularly calcium oxalate and, if present, the crystalline form that it assumes, the presence of starch grains and the shapes they exhibit.

(*e*) The size of the elements. In this respect it must be observed that the *absolute* size may vary according to the age of the trunk, but the *relative* size is always constant; hence, although the absolute size should be determined, greater weight is to be laid upon the relative size of each variety of cell present, when compared with the others.

Although the examination and identification of powdered woods are by no means without interest or importance, the student is advised to refrain from that part of the subject until he has further advanced in his studies.

Quassia Wood

Source.—The quassia wood official in the British Pharmacopœia and used in this country is obtained from *Picræna excelsa*, Lindl. In Germany that obtained from *Quassia amara*, Linn., is also employed.

Quassia wood is one of the most suitable woods for the student to investigate, on account of its relative softness and the ease with which it can be separated into its constituent elements.

Preparation for Cutting

From a small log of quassia wood saw off a disc about 2 cm. thick. Split from this disc, by cuts passing through its centre, one or more wedge-shaped pieces, and remove the bark; the wood will present somewhat the appearance of fig. 34.

The surface of this piece of wood that is transverse to the long axis of the log is the transverse surface, and any section taken from that surface, or parallel to it, is a transverse section (fig. 34, *tr*).

The surface exposed by cutting the disc downwards through the centre is a radial surface, because the cut is coincident with a radius of the disc. A section taken from this surface, or from any surface exposed by a radial cut (not by a cut parallel to the radius), is a radial section (fig. 34, *r.*).

The surface exposed by cutting the disc downwards at right angles to the radius is a tangential surface, because the cut is made parallel to a tangent of the disc. A section made from this surface, or any surface parallel to it, is a tangential section (fig. 34, *t*).

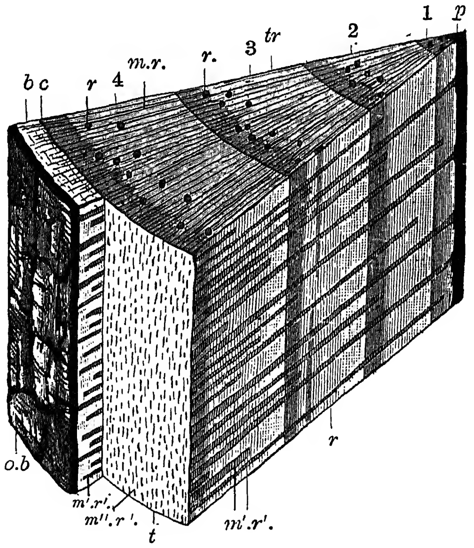

FIG. 34.—Piece cut from a pine stem four years old. 1, 2, 3, 4, the wood produced in the four successive years; *r*, *t*, *tr*, the radial, tangential, and transverse surfaces respectively; *b*, bark; *c*, cambium; *m.r.*, medullary ray on transverse section; *m'.r'.*, the same on radial section both in wood and bark; *m''.r''.*, the same on tangential section; *r*, resin dust (on transverse section); *p*, pith. ×6. (Strasburger, Noll, Schenck, and Schimper.)

Smooth the transverse surface with a sharp penknife, and examine it with a lens. Observe the medullary rays running in the direction of radii; they are sufficient to indicate the nature of the surface exposed (fig. 34, *m.r.*).

Do the same with a tangential surface, taking care, by examining the transverse surface, to see that the tangential surface is exactly at right angles to the medullary rays; if this be not the case, oblique sections will be obtained which are difficult of interpretation. Observe on the tangential surface small elongated-oval markings (fig. 34, *m″.r″.*); these can be traced on the edge of the piece of wood, and can be identified with the medullary rays. Their particular appearance is sufficient to identify the surface as a tangential one.

Next cut a radial surface, taking great care that the cut passes as nearly as possible along a medullary ray; this can be done by comparing the transverse surface, and paring away the wood until the cut passes along a medullary ray. Examine the radial surface, and observe that the medullary rays are evident as narrow bands, easily distinguishable from the remainder of the wood by their different appearance (fig. 34, *m′.r′.*).

The student should not, on any account, fail to make himself familiar with these three surfaces. Having done so, he can cut the piece of wood in varying oblique directions and examine the appearances of the sections. In cutting transverse, radial, or tangential sections of wood, he should also be very particular to have them as nearly exact as possible, as their interpretation is, thereby, much facilitated.

The term 'longitudinal' section is often employed to denote a section parallel to the long axis, and hence either radial or tangential. Since the term lacks precision, care should be taken in employing it.

Separation of the Elements

The student will find it most profitable to begin by separating the wood into its component structural elements, and examining them.

From a radial surface cut with a penknife ten or twenty narrow, thin slips or shavings, some about 1 cm. long, 20 mm. wide, and 2 mm. thick, others thinner. Transfer them to a test-tube half full of nitric acid of specific gravity about 1·3 (two volumes of official nitric acid diluted with one of water answers very well); add to this from 1 to 2 grammes of potassium chlorate in crystals, and warm gently until evolution of gas commences. The pieces of wood first darken in colour and then bleach. Let the action continue, maintaining a gentle evolution of gas by warming, if necessary, from time to time, until glistening fibres are seen to be separated from the pieces of wood when the tube is gently shaken. Stop the action by carefully pouring the whole of the contents of the tube into a beaker of water. From this transfer the fragments of bleached wood by means of a glass rod to fresh water, and finally to alcohol. In this they should remain a little time (if possible a few hours) to remove the gas that accumulates in the cells.

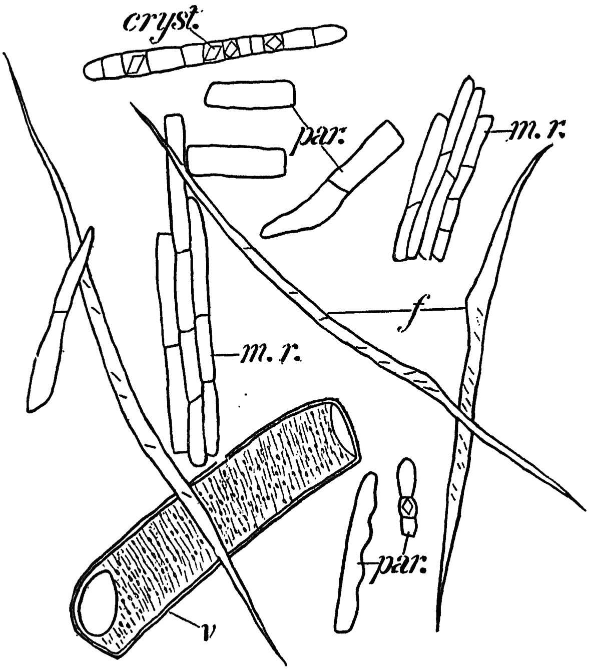

Fig. 35.—Quassia Wood. Elements isolated by potassium chlorate and nitric acid. *cryst.*, the membranes in which calcium oxalate crystals have been enclosed; *f*, wood fibres; *m.r.*, cells of medullary ray; *par.*, wood parenchyma; *v*, vessel. ×100.

This process is known as Schulze's maceration process, and the mixture of nitric acid and chlorate of potash as Schulze's maceration mixture (to be carefully distinguished from Schultze's solution, which is chlorzinciodine). The strength of nitric acid and the time necessary for disintegration vary with different drugs, and cannot, therefore, be definitely stated. If the reaction is stopped too soon, the wood will not separate into its elements; if allowed to progress too far, the whole will be oxidised and destroyed.

Take one of the pieces of bleached wood from alcohol, and transfer it to a drop of water on a slide; tease a small fragment with the dissecting needles until it separates into its component elements; cover and examine under the low power. Do not add glycerin, as it makes the cells too transparent.

Cells of the following forms will be found:

(*a*) *Wood fibres* (fig. 35, *f*), conspicuous by reason of their length and tapering ends. Observe the width of the cell, the thickness of the wall, and the scattered oblique pits (not always easily seen, especially if the light is too strong).

(*b*) *Wood parenchyma* (fig. 35, *par.*), recognisable by the oblong shape of the cells and the rounded pits in the walls. They often contain delicate membranes that present a quasi-crystalline appearance; these are the membranes that have enveloped calcium oxalate crystals (see below). Sometimes several cells adhere end to end, the terminal ones being bluntly conical. Such a row of cells is developed from a single cambial cell by transverse division, and illustrates the formation of wood parenchyma.

(*c*) *Cells of the medullary ray* (fig. 35, *m.r.*). These closely resemble the wood parenchyma; but they occur in plates of cells more often than in single rows, and, when they adhere to wood fibres, cross these at right angles; indeed, their position constitutes the sole means by which they can be distinguished from wood parenchyma.

(*d*) *Vessels* (fig. 35, *v*). Although these are by no means so numerous as the wood fibres, wood parenchyma, or cells of the medullary rays, they are not difficult to find. They are readily identified by their much larger size, cylindrical shape, and numerous pits. On careful examination, the remains of transverse walls with large perforations can often be distinguished.

The student should now sketch two or three of each kind of cell.

The most careful search will not disclose any other cell forms, and quassia wood is therefore built up of these elements. It remains to be seen how they are arranged.

Preparation and Examination of Sections

Radial Sections.—Soak a wedge of the wood in water for twelve hours or, as only traces of starch are present, boil it for fifteen minutes. Smooth the transverse surface with a penknife, and examine it with a lens; make certain that the radial surface is exactly radial; if it is not, trim it with a knife until it is. Make on the radial surface a pencil mark

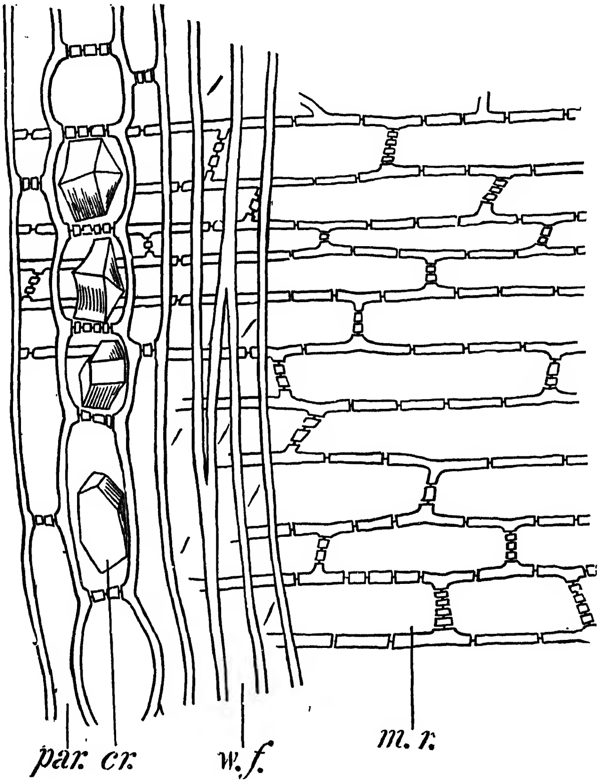

Fig. 36.—Quassia Wood, radial section. *cr.*, crystals of calcium oxalate; *m.r.* medullary ray; *par.*, wood parenchyma; *w.f.*, wood fibres. ×320.

about 3 mm. square, or rather less. Split the wedge, and cut out the pencil mark to a depth of 5 mm. so as to obtain a rectangular fragment, the small end of which bears the pencil mark. Fix this fragment in pith, so that the pencil mark is level with the surface. Cut several sections, rejecting the first two or three. Be careful to draw the edge of the razor, from heel to point, across the wood, and avoid any motion similar to that used in sharpening a pencil.

Transfer the sections, as cut, to spirit, in which they should remain for several minutes; mount one in dilute glycerin (equal parts), taking care to spread the section out with the needles, if necessary. Cover and examine. If the section contains many air bubbles, warm it until the liquid gently boils, and cool.

Identify on this section (or others if necessary) all the elements that have been previously observed and sketched.

The *medullary rays* will be conspicuous as broad bands of cells extending along the entire section, provided that it has been truly radial; most of these cells are elongated in the direction of the radius.

The *wood fibres* are easily found. They are long and narrow, and assume a direction at right angles to the medullary rays. The tapering ends are not easy to see, as the fibres interlace; hence the dissociation treatment is valuable, as it is the only means by which the shape of the fibre can be accurately ascertained.

The cells of the *wood parenchyma* are axially elongated; they are not so numerous as the wood fibres; from the cells of the medullary rays they are distinguished by their axial (not radial) elongation.

The *vessels* are very large, but only a portion of the pitted wall can, as a rule, be found; large spaces between the wood fibres indicate the position of the vessels, the walls of which are often cut away. Examine the pits carefully; they are bordered.

Observe, in the cells of medullary rays and wood parenchyma, large crystals of calcium oxalate.[1] They are enclosed in delicate membranes, which are invisible until the crystal is dissolved away, when they are left behind (compare the examination of the dissociated cells).

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[1] It must be observed that in some specimens of Jamaica quassia wood calcium oxalate crystals are difficult to find.

Observe also occasional small grains of starch (iodine reaction).

Sketch a small portion of the radial section, taking care to include part of the medullary ray.

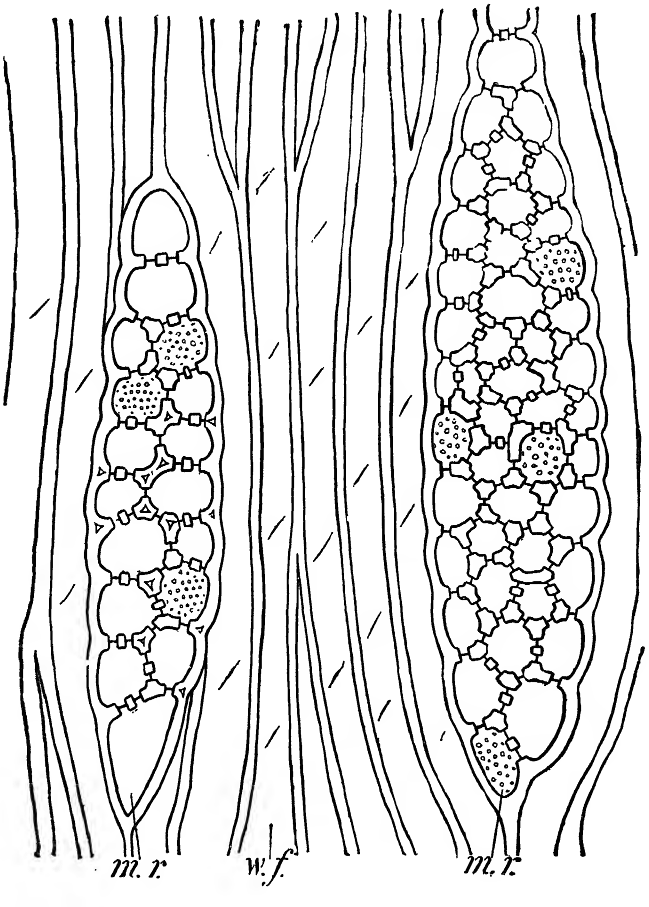

FIG. 37.—Quassia Wood, tangential section. *m.r.*, medullary rays (the pits in the walls of the cells are rather too conspicuous); *w.f.*, wood fibres. × 320.

Tangential Sections.—In the same way prepare a piece of wood for tangential section, taking care, by examining with a lens, that the surface marked is really the tangential surface.

Observe in this section wood fibres, wood parenchyma, and vessels, presenting the same appearance that they did in radial section. The medullary rays, on the other hand, are cut transversely to their length, whereas in the radial section they are cut parallel to their length. The tangential section, therefore, exhibits the height and breadth of the medullary rays, which take the form of elongated oval groups of cells inserted between the wood fibres. Count the average number of cells in the length and greatest breadth of the rays. Beyond this do not examine the section further.

Sketch a portion of the section.

Transverse Sections.—Lastly, cut in a similar manner transverse sections. Examine first the medullary rays. These exhibit their length, but the breadth is seen only at the particular point at which they happen to have been cut (compare the tangential section); hence they are mostly two or three cells wide, but occasionally as many as four (middle of a large ray) or as few as one (upper or lower extremity of a ray). Observe that the cells appear radially elongated.

Next examine the vessels. Their large size makes them conspicuous. They are usually in small groups of two or three, often extending from one medullary ray to the next.

The remainder of the wood must be composed of wood fibres and wood parenchyma, since no other elements are present. These are, at first, not very easily distinguished from one another.

The wood fibres have thick walls; the cells are polygonal in outline, often varying much in size, according to the point in their length at which they have been cut; they do not exhibit pits or any transverse walls with pits.

The wood parenchyma cells have comparatively thin walls, are of more uniform size, and more regularly rectangular; they often exhibit pits, and sometimes crystals. They occur mostly in groups stretching from ray to ray, and often form irregular rings on the section of the trunk (false annual rings).

Sketch a small portion of the section.

Take a fresh section, transfer it to a drop of water on a slide; remove the water by filter paper, add one or two drops of solution of phloroglucin, and cover with a watch-glass. After two or three minutes (longer for delicate experiments) remove the phloroglucin solution by filter paper, add a drop of strong hydrochloric acid, cover with a coverslip, and examine.

All the cell walls should have assumed a deep crimson red colour, an indication that they are lignified. The calcium oxalate is dissolved by the acid; the membranes in which the crystals were enclosed remain, and in favourable sections (tangential or radial) may be seen also to be lignified.

The student should now sum up the results of his investigation as follows:

(1) The only elements present are wood fibres, wood parenchyma, vessels and cells of medullary rays.

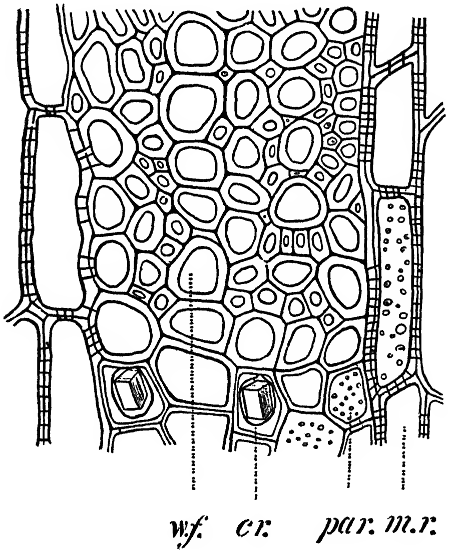

FIG. 38.—Quassia Wood, transverse section. *cr.*, crystals of calcium oxalate, enclosed in delicate membranes; *m.r.*, medullary ray; *par.*, parenchyma of wood; *w.f.*, wood fibres. ×320. (After Meyer.)

(2) The medullary rays are four to twenty cells high and one to four cells wide; they are mostly ten cells high and three cells wide. The vessels are sometimes single, more often in groups of two or three; the wood parenchyma is mostly in bands extending from ray to ray.

(3) The cell walls are all lignified. The wood parenchyma and medullary rays contain calcium oxalate in single crystals, and, here and there, a little starch.

(4) The wood fibres are long and taper gradually to fine points; they have moderately thick walls.

A comparison of the anatomical structure of the official quassia wood with that of Surinam quassia will illustrate the value of these characters. Surinam quassia has medullary rays that are mostly one cell wide, and is free from calcium oxalate; the official (Jamaica) quassia has medullary rays from one to four cells wide, and contains calcium oxalate.

Other Methods for separating Tissues into their Elements

Although Schulze's maceration mixture is the method usually adopted, it is by no means the only one, nor is it in all cases the best.

Isolation by Chromic Acid.—A solution of 10 per cent. of chromic acid in dilute sulphuric acid forms a very useful oxidising mixture, capable of destroying the middle lamella and thus isolating the cells. It is best used as follows:

Cut several sections (preferably radial and not too thin) and immerse them in a watch-glass containing twenty to thirty drops of the solution. From time to time transfer a section with a glass rod to a drop of water on a slide, wash with one or two drops of water, and tease it a little with the needles or press it with a glass rod. As soon as the trial section easily separates into its elements, transfer the remaining sections carefully to a dish of water. Mount one in a small drop of water and cover with a coverslip; a little pressure on the coverslip, accompanied by a sliding movement, will bring about the separation of the cells.

The time required varies with the object and temperature; from ten to thirty minutes often suffice.

Isolation by Mangin's Method.—Mangin's method of separating the cells is based upon his belief that the middle lamella consists of calcium pectate. The sections are first digested for forty-eight hours in a mixture of alcohol (4 volumes) and hydrochloric acid (1 volume); this dissolves the calcium as chloride, leaving the pectic acid undissolved. The sections are then digested in a 10 per cent. solution of ammonia or ammonium oxalate, which removes the pectic acid as pectate. The middle lamella having been thus dissolved, the cells separate.

This method is not so suitable for woods as either the nitric acid or chromic acid method.

Richter's Method.—Richter has shown that a strong solution of ammonia answers a similar purpose, but it requires a much longer time (14 days) and is more suitable for parenchymatous tissue than for wood.

Vétillard's Method.—This depends upon the action of boiling 10 per cent. solution of sodium carbonate, and has already been described in Section II. It is specially suitable for the purpose there indicated.

Digestion with Potash.—Isolation by caustic potash is particularly well adapted for parenchymatous tissues (leaves, &c.) but not for wood. Fragments of the objects are digested in water containing from 1 to 5 per cent. of caustic potash, in a water-bath, for ten to thirty minutes; they are then washed free from alkali with distilled water, and the cells separated by teasing with the needles. The treatment with caustic alkali is liable to produce swelling of the cell wall, which has always to be taken into consideration.

Putrefaction.—By this means the layers of cells in the pericarp of the wheat and other grains can be loosened and separated. The grains are crushed, mixed with water, and set aside in a warm place (20° to 30° C.) for several days. The layers are then separated by teasing and stripping.

Tests for Lignification

Phloroglucin.—The best test to detect lignification of the cell wall is that by phloroglucin and hydrochloric acid, as described on p. 62. The following, however, are useful, and the student should make himself familiar with them.

Chlorzinciodine.—This reagent is best applied by mounting a section in water (without the coverslip), removing the water as completely as possible by filter paper, dropping a drop of the reagent upon the section, covering, and examining. While cellulose assumes a bluish or violet colour with the reagent, he lignified cell wall is stained yellow. If, however, the extent of the lignification is slight, the yellow colour may be overpowered by the blue cellulose reaction.

Iodine and Sulphuric Acid.—The mode of using these reagents has already been described (p. 26); the lignified cell wall assumes a yellow colour.

Aniline Hydrochloride, with or without hydrochloric acid, colours the lignified wall yellow, and is useful when the presence of acid is objectionable.

The substances that produce these colour reactions are destroyed by strong oxidising agents, and are removed by prolonged digestion with caustic alkalis; hence the isolated elements, after maceration with nitric acid and potassium chlorate, or with caustic potash, may not give the lignin reaction. Many other reagents for the detection of lignification of the cell wall have been proposed, but none of them have come into such general use as the phloroglucin test.

Removal of Air from Sections

Air bubbles frequently occur in microscopical preparations, sometimes in small, sometimes in large numbers. In the former case they seldom materially interfere with the examination of the preparation, but in the latter their removal is often necessary. They may be recognised by the wide black margin surrounding a brilliant centre. When lying free in the mounting medium they appear circular, but in cells they usually fill the cavity and assume the outline of the cell.

One of the following methods may be adopted for removing them:

(1) Transfer the sections to strong alcohol, and allow them to remain in it until freed from air; the time necessary for this varies from fifteen minutes to twelve hours. The method is usually successful, but takes time, and has further the disadvantage that all cell contents soluble in alcohol, such as volatile oil, resin, &c., will be dissolved, and possibly other changes induced. If the structure only of a drug is to be studied, this is seldom objectionable, but, for the examination of the cell contents, other means of attaining the object must be adopted.

(2) Heat the sections in water, dilute glycerin, or other medium until the liquid boils; keep it gently boiling for a few seconds, then cool. This method also entails alteration of the cell contents, and not unfrequently alteration of the cell wall, which has a tendency to swell under these conditions. It is, however, effectual and often resorted to, especially for sections of hard tissues,

which may be thus treated on the slide (covered with a coverslip).

(3) Place the sections in a dish of recently boiled and cooled distilled water; allow them to remain until the water has dissolved the air, for which usually several hours are necessary. The method is very effectual, and entails but little change in cell walls or cell contents, hence it is in many cases preferable to the first two methods. Care must be taken to boil the water for ten minutes, to cool it thoroughly, and to use it in abundance, say from 25 to 50 cubic centimetres for a dozen sections.

(4) Place the sections in a small dish of water under the receiver of an air-pump, and exhaust the air. By this means the air is rapidly and effectually removed from the sections; unfortunately, an air-exhaust is not always at hand, otherwise this would probably prove the best and easiest means of getting rid of air.

The student should be careful to bear in mind the disadvantages of the various methods enumerated, and to select that method which will best answer for the particular section to be treated.

Guaiacum Wood

Source.—The official guaiacum wood is the heart wood of *Guaiacum officinale*, Linn., and of *G. sanctum*, Linn.

Preparation.—On account of its extreme hardness, guaiacum wood is not easy to cut; it requires prolonged soaking in water or dilute glycerin. The structure may be equally well determined by the examination of the pale sapwood or of small fragments that have been boiled for fifteen minutes in solution of potash, but in these cases the localisation of the resin cannot be ascertained.

The sections may be examined in glycerin. The elements may be separated by macerating radial sections in chromic acid.

Description.—The transverse section shows that the bulk of the wood consists of very strongly thickened wood fibres, which are often cut more or less obliquely, due to the varying course which they take in the stem. In section they appear polygonal or nearly rounded. Isolated by chromic acid (or other means) they are seen to be mostly of moderate length (400 to 600 μ) and very thick-walled; they either taper gradually to a fine point or are abruptly narrowed towards the end, not unfrequently forking at or near the extremity. The pits are scattered clefts, and are numerous.

The vessels are mostly large and isolated, seldom two or three together. They have thick, pitted walls, and are often filled with yellow resin. In surface view the walls are seen to bear very numerous small pits.

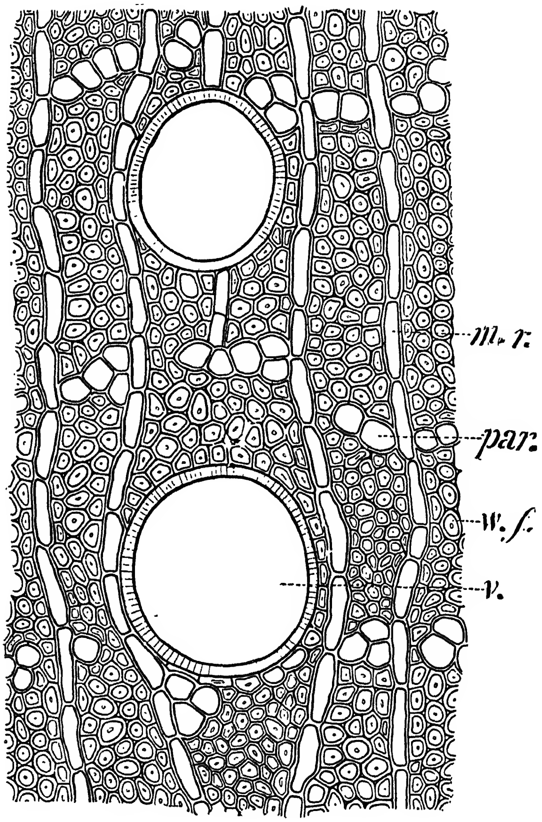

FIG. 39.—Guaiacum Wood, transverse section. *m.r.*, medullary ray; *par.*, wood parenchyma; *v*, vessel; *w.f.*, wood fibres. × 320. (After Berg.)

The wood parenchyma cells are arranged in narrow tangential bands, usually one cell wide, but sometimes two or three; such bands often stretch continuously from one medullary ray to the next, and so form false annual rings.

In these cells calcium oxalate crystals are by no means uncommon.

The medullary rays are one cell wide and (in tangential section) from four to six cells high; they often assume a sinuous course, deviating to allow room for the vessels. The cells are pitted, and are strongly elongated.

The vessels, wood parenchyma, and medullary rays of the heart wood are filled with yellow or brown resin, which also seems to permeate the walls of the wood fibres and even be present in the cavity. In the cells of the wood parenchyma a large crystal of calcium oxalate can occasionally be found.

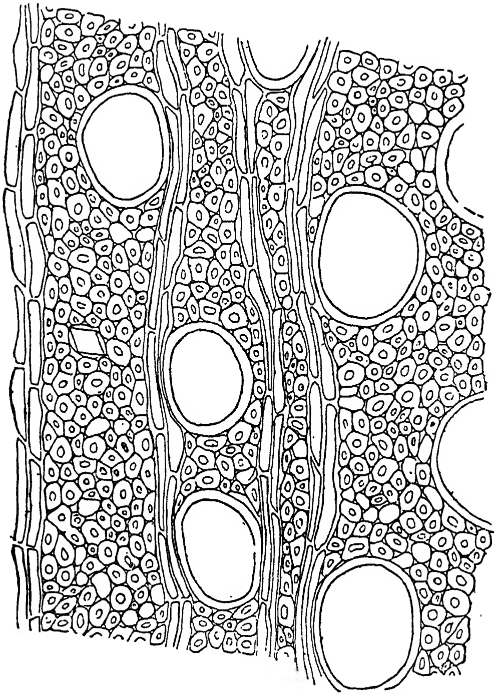

Fig. 40.—Yellow Sandal Wood, transverse section. ×200. (Petersen.)

Yellow Sandal Wood

Source.—Yellow sandal wood is the heart wood of *Santalum album*, Linn.

Description.—The medullary rays are from one to three, often two, cells wide and about six high ; the cells are strongly elongated, and have large pits.

The wood consists principally of thick-walled wood fibres. The vessels are mostly isolated and large, varying from 50 to 80 μ in diameter. The wood parenchyma is small in quantity ; it is distributed in tangential or oblique groups of from two to five cells, not often long enough to extend from one medullary ray to the next. Here and there a large crystal of calcium oxalate can be seen ; in radial or tangential sections, axially extended rows of from ten to fifteen such crystal cells can be found.

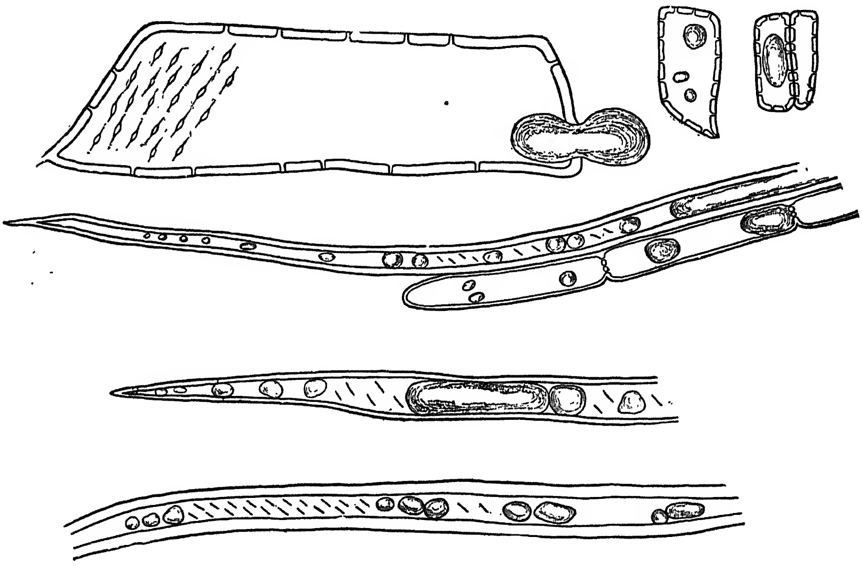

FIG. 41.—Yellow Sandal Wood. Elements isolated by chromic acid and showing the volatile oil (in the vessel some only of the pits have been introduced). × 300.

Thin longitudinal slices (or rather thick sections) can readily be separated into their constituent elements by maceration in chromic acid solution. After replacing the reagent by water the elements can be examined. Globules of oil (which stain with alkanna or osmic acid) are to be seen in all of them, but the wood parenchyma and medullary rays appear to contain most. The oil dissolves readily in alcohol (distinction from most fixed oils), and is doubtless the volatile oil that imparts to the wood its characteristic aroma. It is remarkable that in this case the oil is not secreted in special cells, but is found in all the constituent elements of the wood.

The wood of *Fusanus acuminatus*, R. Br. (South Australia), is distinguished from that of *Santalum album* by the arrangement of the vessels; these mostly form radially extended groups of from two to five. The wood contains but little calcium oxalate, and, in tangential section, the medullary rays often exhibit an elongated terminal cell which adjoins a similar cell belonging to a ray above or below.[1]

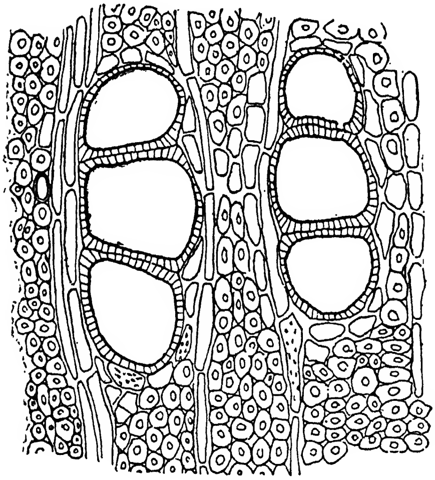

FIG. 42.—Wood of *Fusanus acuminatus*, transverse section. × 200. (Petersen.)

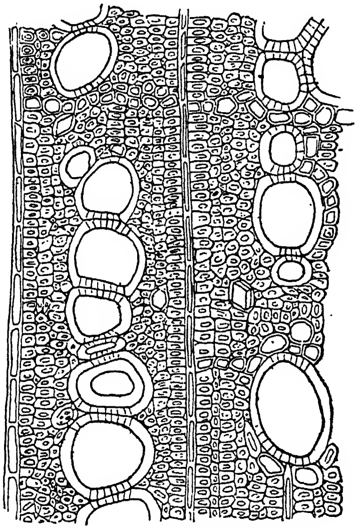

FIG. 43.—Venezuelan Sandal Wood, transverse section. × 200. (Petersen.)

The wood of *Amyris balsamifera*, Linn. (Venezuelan Sandal Wood) exhibits vessels arranged in long radial rows, and both these and the wood parenchyma contain a yellow resin, but only a little volatile oil can be detected by osmic acid.

The student should compare figs. 42 and 43 with fig. 40, and observe attentively the differences in structure which enable these woods to be easily distinguished from one another.

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[1] Petersen, *Pharm. Journal* [3], xvi. 757.

Pine Wood

From a fragment of pine wood (ordinary firewood will do) cut radial sections; transfer them to alcohol; mount one in dilute glycerin and examine.

Observe the large bordered pits on the radial walls of the tracheids.[1]

Cut and examine tangential sections; the large pits previously seen in surface view are now mostly seen in section, the tangential walls bearing smaller pits. Observe particularly the section of the pit (compare the section of a bordered pit in the text-book of botany).

Disintegrate a little of the wood by maceration with potassium chlorate and nitric acid; with the exception of the medullary rays, the wood consists entirely of tracheids.

From a little pine sawdust sift some of the finest powder. Examine in dilute glycerin. The pits are easily seen and identified; some present their surface view, others their section.

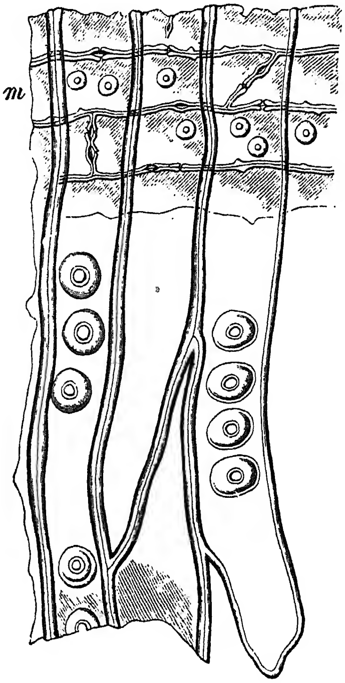

FIG. 44.—Bordered Pits of Fir. *m*, medullary ray. (Tschirch.)

The student should not fail to make himself familiar with the microscopical characters of sawdust, as it is often found in small quantity in vegetable powders, not necessarily, however, as an adulteration (compare also fig. 208, p. 359).

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[1] In the illustration similar bordered pits are seen on the cells of the medullary ray. That is the case with the outer rows of cells of the medullary ray of the fir, but the spruce fir has simple pits and the cells are all alike; in the pine there are two kinds of cells, the outer being distinguished by the very irregular jagged outline of the secondary thickenings.

Calcium Oxalate

Calcium oxalate is of very frequent occurrence in foods, drugs, and vegetable substances generally. Being practically insoluble in water, or in the feebly acid cell sap, it is met with in the solid and more or less definitely crystalline form. It exhibits a considerable variety, both in the shape and size that the crystals assume, but these remain fairly constant for one and the same plant, and not unfrequently genera, or even natural orders, are characterised by the more or less regular occurrence of calcium oxalate crystals of particular form. It acquires, therefore, high importance as a valuable means of identifying a drug. During the process of pulverisation to which drugs are often subjected, many of these crystals, especially if they are of small size, remain intact, and serve therefore to characterise the powder just as they characterise the entire drug. Large crystals are often more or less broken during pulverisation, but, even in this case, there is little difficulty in detecting the broken fragments, and recognising in them portions of larger crystals.

Calcium oxalate is a dimorphous salt, forming either crystals belonging to the tetragonal (quadratic) system and then containing three molecules of water of crystallisation, or crystals belonging to the monoclinic system and containing one molecule of water of crystallisation. Examined in polarised light, the latter appear, when the Nicol's prisms are crossed, brilliantly white on a dark ground; quadratic crystals and cluster (rosette) crystals are less strongly illuminated. Hence examination in polarised light is often of service in detecting minute crystals of calcium oxalate, but it must be remembered that starch grains, fragments of cell walls, crystals belonging to the hexagonal system, &c., will also appear bright on a dark ground.

Although calcium oxalate is one of the most frequent of the crystalline contents of the cell, it is by no means the only one, and the student must be careful to determine the nature of any crystals that may be found, before reporting them as calcium oxalate. This is particularly necessary when examining an unknown drug.

The following are the chief reactions that characterise this compound:

(1) It is insoluble in acetic acid, but soluble in hydrochloric acid without effervescence (distinction from calcium carbonate).

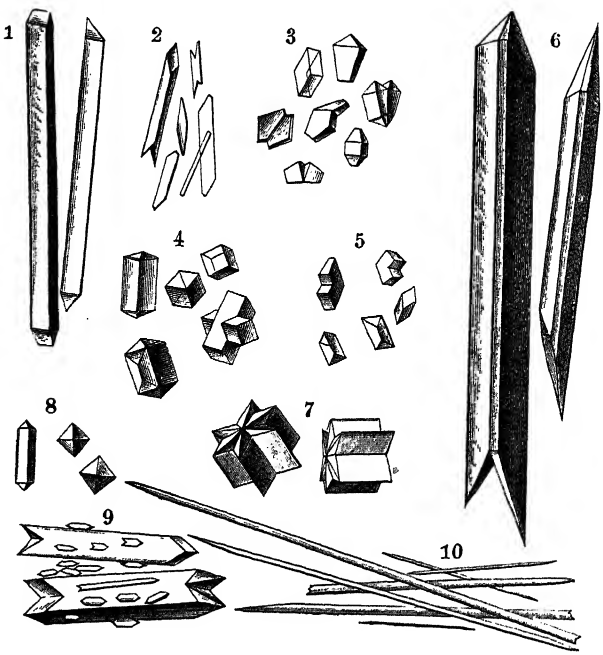

FIG. 45.—Various Forms of Calcium Oxalate Crystals. 1, from cusparia bark; 2, rhatany root; 3, liquorice root; 4, henbane leaves; 5, quebracho bark; 6, orris rhizome; 7, leaf of *Pavonia sp.*; 8, leaf of *Convolvulus arvensis*; 9, quillaja bark; 10, squill bulb. ×250. (Vogl.)

(2) In contact with concentrated, or moderately concentrated, sulphuric acid it is decomposed; the oxalate disappears, while acicular crystals of calcium sulphate make their appearance in the immediate neighbourhood, or at a little distance, according to the current in the mounting medium at the moment when the reaction took place.

(3) Solution of barium chloride does not react with them (distinction from calcium sulphate, which is occasionally

found; crystals of this substance would become encrusted with barium sulphate).

The student should, however, remember that many reagents attack calcium oxalate, especially if the latter is present in comparatively small quantity, and the temperature is elevated. Thus the digestion with solution of caustic potash, which is often resorted to for separating the elements of vegetable tissues, may produce a marked effect on the crystals of calcium oxalate, partially dissolving them, with the production, doubtless, of potassium oxalate and calcium hydrate. Prolonged heating with solution of chloral hydrate often produces a similar effect.

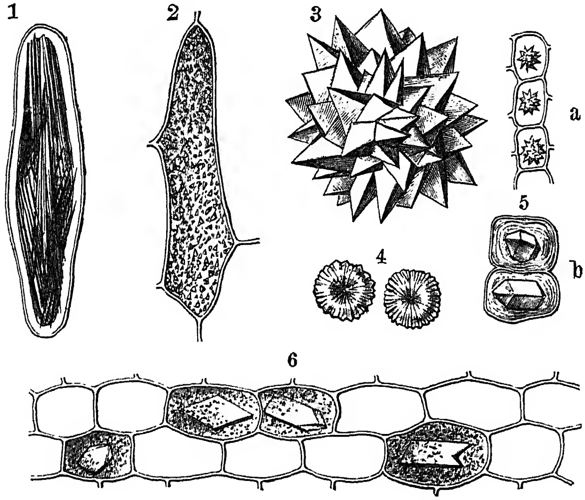

FIG. 46.—Various Forms of Calcium Oxalate Crystals. 1, raphides; 2, sandy crystals (belladonna root); 3, rosette or cluster crystal (rhubarb rhizome); 4, rosettes (argel leaves); 5, *a*, rosettes; *b*, single crystals (horse-chestnut bark); 6, single crystal embedded in sandy crystals. × 160. (Vogl.)

To the fundamental differences in the composition and crystalline form of calcium oxalate must be added differences that are produced by other influences at present not well understood, as, for instance, the rapidity of the currents in the cell sap, the degree of acidity of the latter, the relative amount of calcium present, and so on. These causes all tend to produce a great diversity in the shape, size, &c., of the crystals that are formed, and the latter acquire, therefore, a high diagnostic value. As the great majority of plants contain calcium oxalate in some form or other, its absence (foxglove leaves, lobelia herb) is also a valuable indication of identity.

The forms assumed by the crystals of calcium oxalate may for convenience be classified as follows:

Single crystals.
Aggregates of crystals (rosette or cluster crystals).
Sphæro-crystals (very rare).
Groups of acicular crystals (raphides).
Sandy crystals.

Single crystals are exceedingly common. They may occur singly, or two or more together in the same cell. They may belong to the tetragonal or to the monoclinic system, although the latter are more common. Not unfrequently twin crystals are formed.

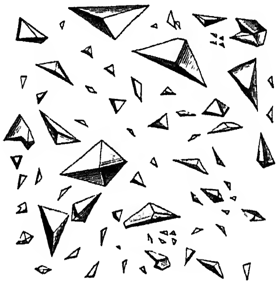

FIG. 47.—Sandy Crystals. ×450. (Vogl.)

Raphides are bundles of long acicular crystals belonging to the monoclinic system. They are very common in monocotyledonous plants, and are often embedded in mucilage. Very frequently the cells containing these crystals are arranged in axial rows.

Rosettes of calcium oxalate are very widely distributed. They vary considerably, both in size and appearance. Apparently they may belong either to the quadratic or monoclinic system.

Sphæro-crystals are closely allied to the rosettes, but their occurrence is rare.

Sandy crystals are very common in Solanaceæ and some other natural orders. The crystals are so minute that their crystalline form is not easily ascertained; they appear, however, to be hemihedral crystals belonging to the quadratic system. Great numbers of these crystals occur in a single cell, completely filling it, and appearing, for optical reasons, as a dark patch that might be mistaken for dust or dirt.

Single crystals and rosettes are frequently found surrounded by a membrane of lignified cellulose. This membrane retains the shape of the crystal even after the latter has been dissolved and removed, and it can be stained by suitable reagents. Sometimes the membrane is easily seen, but sometimes it is so thin as to be invisible until stained.

SECTION VI

STEMS

INTRODUCTION

Under this heading the structure of the stems of some of the official herbs will be considered. These consist of a ring of wood enclosing a pith and surrounded by the bast, cortex, and epidermis. In addition, therefore, to the elements of the wood, these organs will contain epidermis (or possibly the cork that has taken its place), cortex, bast, and pith.

The epidermis usually consists of a single row of cells that vary considerably in size and shape, though they commonly exhibit tangential elongation in transverse sections and axial elongation in longitudinal sections. It generally bears stomata, as well as hairs of varying nature, both simple (protective) and secreting (glandular), and is covered with a cuticle. The characters of this tissue will be more fully described in Section VII, and the foregoing remarks may, therefore, suffice for the present.

Following upon the epidermis is the cortex, the inner limit of which is the endodermis. The cortex is generally composed of parenchymatous cells which, in transverse section, are tangentially elongated near the epidermis, but become more or less isodiametric near the endodermis, and generally exhibit intercellular spaces. That part that abuts upon the epidermis is often collenchymatous—that is to say, the cells exhibit a considerable thickening, especially at the angles; such collenchymatous tissue, however, seldom assumes the character of true typical collenchyma, in which the cells are thickened only at the angles.

If the leaves are small, and the stem takes over, in part at least, the functions of the leaves, then palisade tissue may be developed below the epidermis.

Although the parenchymatous cells of the cortex do not usually exhibit much variation in different plants, yet this tissue often contains other cell forms or special cell contents that may materially assist in the diagnosis of a drug or its powder. Among such the following may be noted as of frequent occurrence:

I. Cell forms—
  (*a*) Palisade.
  (*b*) Sclerenchymatous cells.
  (*c*) Sclerenchymatous fibres.
  (*d*) Internal hairs.
  (*e*) Oil cells.
  (*f*) Oil glands.
  (*g*) Laticiferous cells.
  (*j*) Laticiferous vessels.

II. Cell contents—
  (*a*) Chlorophyll.
  (*b*) Calcium oxalate.
  (*c*) Calcium carbonate.
  (*d*) Starch.
  (*e*) Tannin, &c.
  (*f*) Oil, &c., in special cells or tissues.

These various cells and cell contents will be noticed in detail in Section VII. For the present, the student should confine his attention to such as present themselves in the drugs examined.

In many stems, especially young stems, the endodermis can be distinguished; it may be identified by the following characters:

(*a*) The shape of the cells; in transverse section they are usually rectangular, strongly tangentially elongated, and exhibit no intercellular spaces.
(*b*) The nature of the walls; these are usually thin, but they are commonly suberised, and often lignified; this is especially the case with the central portion of the radial wall, which usually exhibits a slight local thickening, which is lignified.
(*c*) The contents; the cells of the endodermis are sometimes distinguished by containing an unusual quantity of starch, &c.

Sometimes, however, the endodermis cannot be identified.

The tissue immediately within the endodermis, and abutting directly on it, is the pericycle. This may consist of one or more rows of cells, which may remain parenchymatous, or may develop, wholly or partially, into sclerenchymatous cells or fibres; the latter are especially common, and are often designated primary bast fibres. They may occur singly or in groups, or they may form a continuous ring.

Next to the pericycle is the bast ring.

As the primary bast is usually with difficulty to be distinguished and is not important for present purposes, it may be neglected, and the bast ring may be said to consist of bast rays and medullary rays.

This tissue is very small in the official herbaceous stems, and is of little diagnostic value except in so far as it may contain bast fibres, sclerenchymatous cells, or secreting tissue.

The wood may, if necessary, be examined as detailed in Section V. It is desirable, here, to note that a few abnormally developed woods may contain isolated groups of bast tissue (bast parenchyma and sieve tubes) in the wood. These are termed 'interxylary' bast. The following are the chief natural orders in which this abnormal development occurs:[1]

Vochysiaceæ, Combretaceæ, Myrtaceæ, Melastomaceæ, Lythrarieæ, Onagrarieæ, Cucurbitaceæ, Apocynaceæ, Asclepiadeæ, Loganiaceæ, Gentianeæ, Convolvulaceæ, Solanaceæ, Acanthaceæ, Euphorbiaceæ.

The pith consists of parenchymatous cells, frequently of considerable size, often lignified, and usually thin-walled. It may contain sclerenchymatous fibres, sclerenchymatous cells, or various forms of secreting tissue.

In a number of natural orders groups of bast tissue, occasionally accompanied by fibres, are found on the outer margin of the pith abutting on the wood. These are known as 'intraxylary' or 'perimedullary' bast. The following are the chief natural orders in which this tissue has been observed:[1]

Vochysiaceæ, Malpighiaceæ, Olacineæ, Leguminosæ (Papilionaceæ), Combretaceæ, Melastomaceæ, Lythrarieæ, Cucurbitaceæ, Apocynaceæ, Asclepiadeæ, Loganiaceæ, Gentianeæ, Convolvulaceæ, Solanaceæ, Acanthaceæ, Thymelæaceæ, Euphorbiaceæ.

Diagnostic characters of herbaceous stems are to be sought chiefly in the following particulars:

(1) Epidermis: for details of the particular features of this tissue compare Section VII (Leaves).

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[1] For complete lists see Solereder, *Anatomie der Dicotyledonen.*

(2) Primary cortex and bast; the cell contents, such as calcium oxalate, &c., presence and nature of any secretory tissue, presence of sclerenchymatous fibres.

(3) Wood; any abnormal feature, such as the presence of interxylary bast (compare Section V).

(4) Pith; nature and contents of cells; presence or absence of intraxylary bast.

Lobelia Stem

Source.—Lobelia is the herb *Lobelia inflata,* Linn., cut while in flower and dried.

Preparation of Transverse Sections.—Select from lobelia herb (preferably that which has not been compressed) pieces of stem of medium thickness and still bearing the hairy epidermis. Cut several pieces from 1 to 2 cm. long. Should they be too hard for section-cutting, soften them by soaking for a few hours in water.

Embed one of these pieces in pith, as described for ergot. Cut several transverse sections, taking care that in one portion at least the whole of the tissue from the epidermis to the hollow centre is included; it is not necessary that the section should extend over the entire transverse surface.

Place the sections in alcohol; transfer after a few minutes to water; mount one in glycerin.

Examination.—Observe the ring of wood enclosing the remains of the pith, the stems being usually hollow. The structure of the wood may be determined by treating it as directed for quassia (or by a modification of that method), but this study should, for the present, be deferred and the attention concentrated on the tissues that surround the ring of wood.

Take a fresh section, transfer it to a drop of water on a slide, and spread it out with the needles; remove the excess of water with filter paper, and drop a little chlorzinciodine on to the centre of the section; cover with a coverslip.

Examine with a low power; the wood has stained yellow (lignin reaction), while the major part of the tissue exterior to the wood is coloured violet (cellulose reaction).

Examine this tissue with the high power. Observe, about midway between the epidermis and the wood, a single row of cells that differ in appearance from the parenchyma exterior to it; the cells are more oblong, tangentially elongated, often flattened, and all the walls, or part at least of the radial walls, are stained yellow. This layer of cells is the endodermis, and this, together with the tissue exterior to it up to the epidermis, constitute the cortex. The tissue between the endodermis and the wood is composed of pericycle, bast ring and cambium.

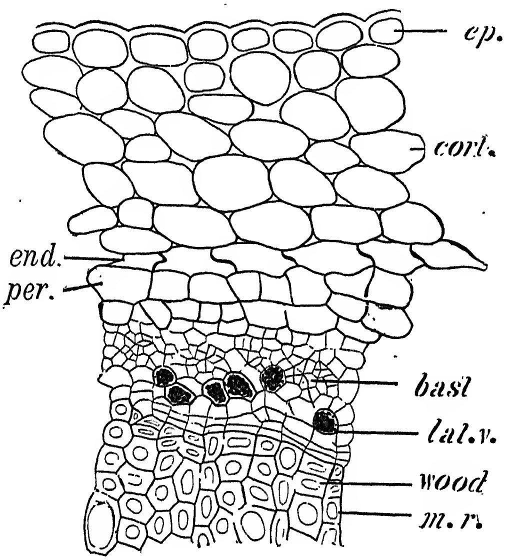

FIG. 48.—Lobelia inflata. Transverse section of stem. *cort.*, cortex; *end.*, endodermis; *ep.*, epidermis; *lat. v.*, laticiferous vessel; *m.r.*, medullary ray; *per.*, pericycle. ×200.

Examine the epidermis more closely.

The cells of which it is composed are quadratic or nearly so (in transverse section). Occasionally hairs may be found arising from it; they are one-celled and bluntly pointed; their walls are not very thick.

Next to the epidermis there follow about six rows of parenchymatous cells; these constitute the cortex; the cells are tangentially elongated and have large intercellular spaces.

The innermost layer of the cortex is the endodermis, the cells of which are closely attached to one another and show no intercellular spaces.

Within the endodermis and abutting immediately upon it is a single or sometimes double row of parenchymatous cells. These are easily distinguished from the cells of the endodermis, which are thin-walled, flattened and lignified, as well as from those of the bast, which are much smaller. They constitute the pericycle, in which there may occasionally be found a cell with a thick lignified wall (pericycle fibre).

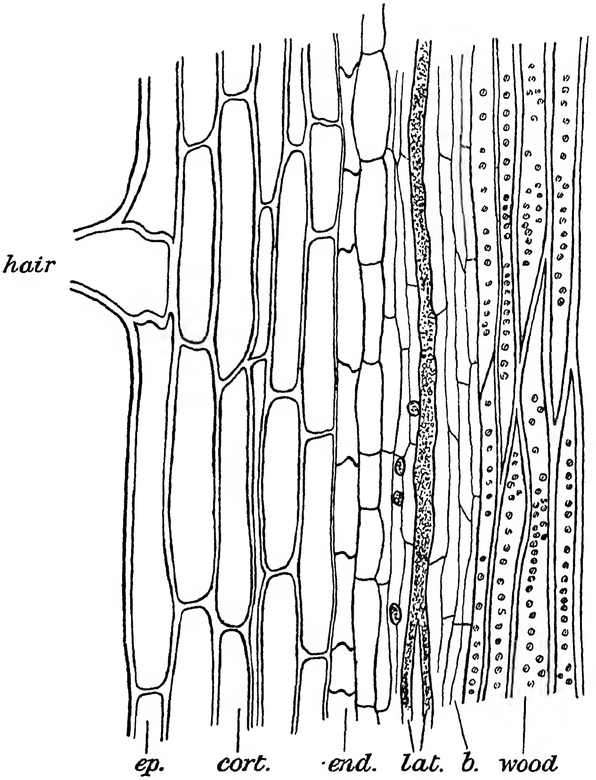

FIG. 49.—Lobelia inflata. Radial section of stem. *lat.*, laticiferous vessel; *b*, bast and cambium. ×200.

Between the pericycle and the wood is the bast (both primary and secondary), but its cells are so small that it is not well adapted for general study.

In the bast observe scattered cells that are conspicuous by reason of their large size and granular contents; the latter have stained deep yellow with the chlorzinciodine. These are evidently special cells containing a particular secretion, and their nature must be ascertained by further investigation.

Preparation and Examination of Radial Sections.—From the lobelia stem cut a small piece not more than 5 mm. long. Split it longitudinally, and embed one half in pith so that the long axis of the lobelia is horizontal and the exposed longitudinal (radial) surface level with the surface of the pith. Hold the pith so that the epidermis of the lobelia is directed outwards. Cut now radial sections, taking care that the razor edge cuts the stem obliquely, passing from the right to the left of the stem as the razor is drawn from heel to point.

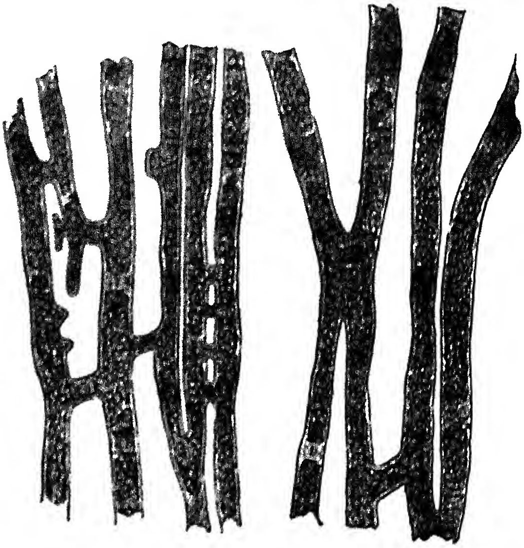

FIG. 50. Lobelia inflata. Laticiferous vessels isolated by potash. × 300.

Treat the sections as directed for transverse sections; mount one in chlorzinciodine.

First identify the endodermis by the flattened shape of the rectangular cells and by their lignified walls. Between the endodermis and the wood observe and examine the elements with granular contents previously alluded to. They are long tubes, which, in favourable sections, may be seen to anastomose with one another.

These elements must be examined still more closely, and this can best be done by isolating them from the surrounding tissues. For this purpose digestion with solution of potash may be employed.

Isolation of Laticiferous Vessels.—Take several pieces of lobelia stem about 1 cm. long; place them in a test-tube half full of solution of potash containing about 1·5 or 2 per cent. of caustic potash. Keep them in a water-bath for about half to one hour. Test one from time to time, to ascertain if the cortex can easily be stripped off and teased out. When this is the case, pour off the alkaline solution and wash with distilled water. Transfer a piece to a slide, and, with the needles, strip from it the whole of the tissue exterior to the wood, taking special care to remove everything down to the wood. Wash this tissue with water to remove adhering alkali, and mount in diluted solution of iodine in potassium iodide.

Examine under a low power. Search among the parenchymatous cells for these peculiar tubes, and identify them by their contents, which are now stained yellow. They freely branch and anastomose with one another; they are laticiferous vessels.

Laticiferous vessels have been found in only a few natural orders, and their presence is therefore highly important as a distinctive feature. The following are the principal orders in which they occur: Papaveraceæ, Olacineæ, Papayaceæ, Compositæ (Cichoriaceæ), Campanulaceæ (including Lobeliaceæ), Convolvulaceæ (Dichondra), Euphorbiaceæ (Hevea, Manihot).[1]

The structure of the wood can, if necessary, be ascertained by treating it as quassia wood was treated. The complete examination of the cortex is best deferred until the student has examined several leaves and made himself acquainted with the methods there given in detail.

Dulcamara Stem

Source.—The stem, about two or three years old, of *Solanum Dulcamara*, Linn.

Preparation and Examination of Sections.—Select a few medium-sized pieces of dulcamara stem, and soak them for a few hours in water; cut transverse sections, transfer them to spirit, and, after a few minutes, from spirit to water.

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[1] For a complete list see Solereder, *Anatomie der Dicotyledonen.*

Mount one in solution of chloral hydrate, spreading out the section, which is liable to curl, with the needles.

Observe the yellowish ring of wood enclosing the remains of the pith and surrounded successively by bast ring, cortex, and cork. Examine the cork carefully.

The cells of which the cork consists are yellowish in colour; they have thin wavy walls that are strongly refractive, and exhibit no intercellular spaces; the consequent sharp contrast with the parenchymatous tissue is characteristic of cork cells. Note their arrangement in regular radial rows, evidently the result of repeated division of the cork cambium cells by tangential walls. The outermost layer bears an intact cuticle, and here and there emergencies or the remains of hairs that have broken off; it is therefore evidently the epidermis, which has not been thrown off. In this plant the epidermal cell itself divides by a tangential wall; the outer half is persistent; the inner half becomes the phellogen, and forms several successive rows of cork cells externally, and subsequently one or two rows of phelloderm cells internally. All these cells are arranged in regular radial rows, a result of their origin. The cork cells can be distinguished by their strongly refractive walls, which also yield the reactions characteristic of suberised membranes (see below). The cells of the phellogen and phelloderm respond more or less distinctly to the tests for cellulose (bluish violet with chlorzinciodine). Those of the phellogen are, naturally, situated between the phelloderm and the cork, and hence may be distinguished by their position. The phelloderm cells have also walls that are thicker than those of the phellogen. The cortex (primary cortex) may be distinguished from the phelloderm by the fact that its cells do not exhibit the regular arrangement that characterises the phelloderm. If, however, much phelloderm is produced, then its cells commonly lose their radial arrangement and become undistinguishable from the cells of the cortex.

The endodermis cannot be easily identified, but its position can be judged. Observe some very thick-walled cells with the cavity almost obliterated; they are isolated or arranged in small tangential groups, forming an interrupted ring between cortex and bast. These are sclerenchymatous pericyclic fibres, and the endodermis will be the line of cells immediately exterior to them. In some pieces of stem the endodermis contains a notable quantity of starch, which aids in distinguishing it, but this is not always the case.

Within the ring of fibres is the narrow bast ring, recognisable by the small irregular cells of the bast, many of which appear filled with dark dust ; these will be examined presently. Within the bast ring is the wood, the study of which may be passed over, and within the wood is the remains of the pith, the cells of which are mostly large, rounded and thin-walled, with intercellular spaces.

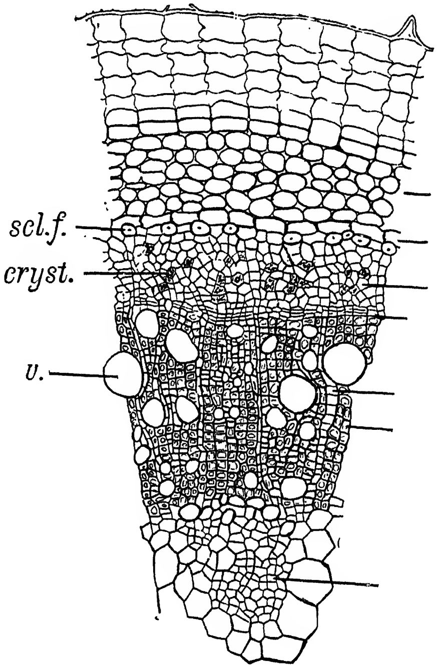

FIG. 51.—Dulcamara Stem. Transverse section, showing intraxylary (perimedullary) bast. × 80.

Examine carefully the ring of pith, especially near the wood ; observe large and small groups of cells, the individual cells of which are smaller than the cells of the pith ; they resemble the cells of the bast. Sometimes an isolated fibre may be found near such a group, and occasionally a cambium has formed. These groups consist of bast tissue, and are intraxylary or perimedullary bast.

Stain a fresh section with phloroglucin and hydrochloric acid. The wood and the cork stain red, and are therefore lignified; the dark dust previously alluded to disappears.

Mount a section in water, focus under the low power, and irrigate with strong sulphuric acid. The parenchymatous cells of the cortex swell and finally disappear; the wood does the same, but more slowly; the cork cells are not affected. Warm gently until the section begins to turn brown; the wood is further destroyed, the cork is still but slightly affected.

Mount a section in water, add a drop of a solution of 10 grammes of chromic acid in 10 c.c. of dilute sulphuric acid. The parenchymatous cells swell and dissolve with evolution of gas bubbles; the wood behaves similarly, but more slowly; the cork is much more slowly affected.

These two reactions are characteristic of the suberised (cuticularised) cell wall, that with sulphuric acid being the most generally useful. Another reaction for the suberised wall consists in warming the section gently with strong (20 per cent.) solution of caustic potash; under these conditions suberised cell walls are disintegrated, and small yellowish oily globules make their appearance. Suberised cell walls are also stained red, but not very deeply, when warmed with solution of Soudan red (see list of reagents).

Isolation of Cells containing Sandy Crystals.—The cells containing the dark granular substance resembling dust must next be more closely examined.

Digest a few pieces of stem in a 2 per cent. solution of caustic potash in a water-bath for about half an hour; wash with distilled water; strip off the cortex and bast, and tease out as directed for lobelia. The cells with sandy crystals are seen now to be mostly long and narrow, and under a high power their contents can be resolved into minute crystals (see fig. 47); they are sandy crystals of calcium oxalate.

Focus a portion of this preparation, or of a radial section, where there are several such cells close together; irrigate with concentrated sulphuric acid very slowly, so as not to create much current. The sandy crystals dissolve and needles take their place; these may remain isolated, or they may form radiating groups. The calcium oxalate is converted into calcium sulphate, which easily crystallises in acicular crystals.

Further proof of the identity of these crystals may be adduced by irrigating a section with acetic acid, in which they are insoluble, and with dilute hydrochloric acid, in which they dissolve without the evolution of gas (which would occur if the substance were a carbonate).

Euphorbia pilulifera

Source.—*Euphorbia pilulifera*, Linn.

Preparation and Examination of Transverse Sections.—Cut a transverse section of the stem of *Euphorbia pilulifera* as directed for lobelia, and mount in glycerin.

The cells of the epidermis are small in transverse section. Those of the cortical parenchyma are larger with small intercellular spaces, and exhibit tangential elongation.

The endodermis cannot be distinguished either by chlorzinciodine or by phloroglucin and hydrochloric acid, or by any difference in the appearance of the cells or their contents.

Rather nearer to the wood than to the epidermis observe groups of cells that differ from the cells of the cortex in appearance and are arranged in a diffuse circle ; they vary in size in different specimens ; they are often compressed into an oval, triangular, or polygonal outline ; sometimes they exhibit a large lumen. The walls are usually very distinctly striated, a feature common to many euphorbiaceous plants. They are pericyclic fibres, and the layer of cells immediately exterior to these fibres is probably the endodermis, although its cells exhibit no characteristic features.

Between the pericyclic fibres (which are often, but less correctly, termed ' bast fibres ' or ' primary bast fibres ') and the wood is a narrow ring of bast, the cells of which are often much compressed, and hence indistinct. In this ring of bast observe some few scattered cells which, in transverse section, appear much larger than the other cells of the bast. Direct attention particularly to these cells.

Stain a section with chlorzinciodine. The pericyclic fibres assume first a pink, then red, finally deep red, brown, or black colour. This might be taken to indicate strong lignification, but a section stained with phloroglucin shows them to be but slightly lignified.[1]

The large cells in the bast are seen, in some cases at least, to contain a granular substance that has coloured yellow.

Isolation of Laticiferous Cells.—Digest one or two fragments of the stem with caustic potash, as directed for lobelia. Tease the tissue of the bast with the needles, and examine in iodine water. The cells with large cavities can now be isolated and identified by their brown granular contents. They are long branching tubes, which show no anastomoses as the similar tubes from lobelia stem did; they are laticiferous *cells*.

Distinguish carefully between laticiferous *cells* and laticiferous *vessels*; the former branch freely, but do not anastomose, the latter exhibit numerous lateral branches which anastomose with neighbouring vessels.

The following are the principal natural orders in which laticiferous cells have been found: Apocynaceæ, Asclepiadeæ, Euphorbiaceæ, Urticaceæ.

Broom Stem

Source.—The young stem of *Cytisus Scoparius*, Link.

Preparation and Examination of Sections.—Cut transverse sections; immerse in spirit, transfer to water; mount one in glycerin. Examine the epidermal cells; they are square or oblong in outline, and possess a thick cuticle. Pass on to the cortex, which is composed of several layers of parenchymatous cells. At intervals the cortex is extended into projections (wings); in each of these wings observe two groups of fibres, one near the epidermis, the other near the wood. The fibres are much thickened, the cavity being almost obliterated; the walls show distinct stratification, but are only slightly lignified (test by phloroglucin and hydrochloric acid).

Between the bundle of fibres in the apex of the wing and the epidermis are one or two rows of parenchymatous cells with thickened sides and angles (collenchymatous).

The endodermis cannot be distinguished by appearance from the other cells of the cortex, but may be identified by its position. Observe the interrupted band of fibres several rows wide near the wood; these are developed from the pericycle, and the row of cells immediately exterior to them is the endodermis.

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[1] The nature of the walls of these fibres requires further investigation.

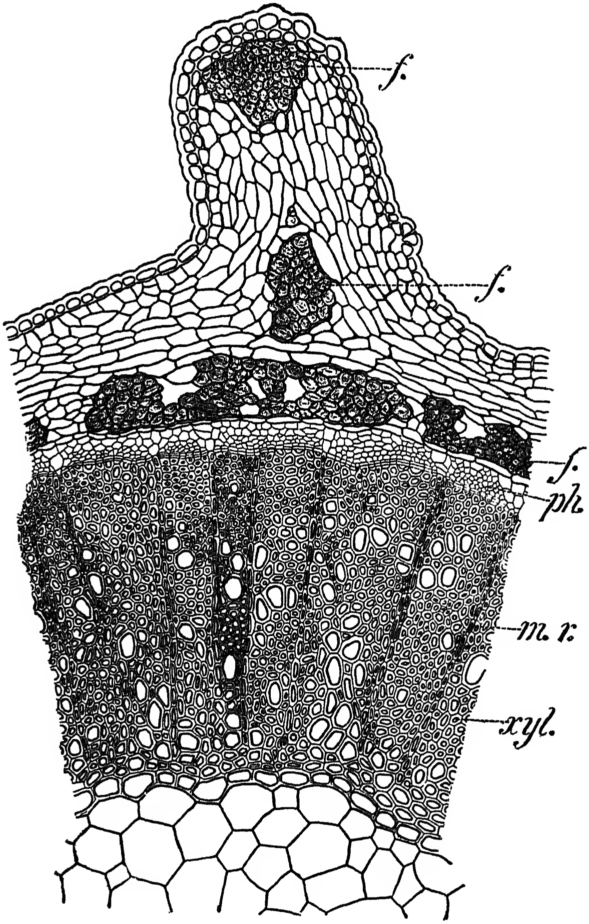

FIG. 52.—Broom. Transverse section of young stem. *f.*, fibres; *m.r.*, medullary ray; *ph.*, phloem; *xyl.*, xylem. × 100.

Examine these fibres; the outline of each is oval or polygonal; the wall is thick and shows stratification; the cavity is reduced to a slit.

Within the ring of fibres is a narrow ring of bast followed by wood. Neither bast nor wood need now be minutely examined.

The pith consists mostly of very large thin-walled cells.

SECTION VII

LEAVES

INTRODUCTION

Although leaves differ in structure and organisation from the axes upon which they are borne, yet their tissues are usually continuous with and analogous to, or even identical with, those of the stems that produce them. The epidermis of the stem is continuous with that of the petiole and lamina of the leaf; the cortex of the stem passes into the cortex of the midrib, of which the mesophyll is but a specially organised development. A portion of the stele leaves the stem and passes into the leaf, where it constitutes the meristele of the midrib and eventually subdivides into the schizosteles of the veinlets.

In discussing the general anatomy of leaves it is advantageous to deal first with the epidermis and its appendages, then with the mesophyll, and lastly with the veins.

Epidermis.—The epidermis usually consists of a single layer of cells, the shape of which varies in the leaves of different species, but is constant in all plants of the same species growing under normal conditions.

In transverse section the epidermal cells usually exhibit a rectangular outline, this being especially the case with those from the interneural spaces, the cells lying above or below the midrib being rounded at the angles and therefore more or less oval.

The cuticle varies in thickness, being generally thick in shrubs (jaborandi) but thin in herbaceous plants (hemlock). It is commonly smooth, but sometimes exhibits projecting ridges (belladonna) or protuberances (coca); in surface view the former appear as striations, the latter as ill-defined circles.

The epidermal cells may appear, in surface view, either comparatively small and straight-walled (buchu, coca, jaborandi), or large, with thin, undulating, or even strongly wavy walls (foxglove, hemlock, henbane, belladonna). In powdered herbs the epidermal cells usually present their surface, not their section, to the observer, and the study of the differences thus exhibited becomes, therefore, extremely important.

Stomata.—Most leaves bear stomata, either upon the upper or lower surface or both, but submerged leaves are usually free from them. When they occur they are not necessarily uniformly distributed over the surface that bears them, although this is the more frequent occurrence, but are sometimes grouped in various ways (bearberry). Very often the cells surrounding the guard-cells of the stomata assume a particular arrangement, which is constant for the same species and often recurs in a number of species belonging to the same natural order. Thus in the coca leaf the stoma is surrounded by four cells, two of which have their long axes parallel to the ostiole; in senna a similar arrangement is met with. In labiate leaves, on the other hand, the stoma is enclosed between two cells the long axes of which are at right angles to the ostiole. In jaborandi there are several narrow cells tangentially arranged round the stoma, while in leaves derived from Solanaceæ and Compositæ there are usually three or four cells, one of which is smaller than the others. Nor are the stomata always inserted at the same level, for while some are elevated above the epidermis, others may be sunk below it, the latter being especially the case with plants growing in arid districts (senna).

Hairs.—Many leaves, including several officinal ones, are glabrous (coca), but it is more common to find hairs either distributed over the lamina or restricted to the veins. These hairs exhibit an infinite variety in shape and nature, but they are constant in the same species, and even different species of the same genus are often found to be furnished with similar hairs. They possess, therefore, very great diagnostic value. Hairs may be divided into two classes, viz. simple (or protective) hairs, and glandular (or secreting) hairs. These two classes may be considered separately.

(*a*) *Simple Hairs.*—These may be either unicellular or pluricellular, according as they consist of a single cell or of several cells. If the cells are arranged in a single row, the hair is uniserial; if in several rows, pluriserial. The length and shape of the hair, the thickness of the wall, and the nature of the surface should be carefully studied; thus in senna the hairs are one-celled, thick-walled, and warty; in foxglove they are uniserial, pluricellular, and the walls are thin and slightly warty, and so on.

(*b*) *Glandular Hairs.*—These are distinguished by their terminating in a cell or collection of cells that secrete resin, volatile oil, &c. The secreting cells are, therefore, borne upon pedicels, which, like simple hairs, may be unicellular or pluricellular, uniserial or pluriserial. Very often the pedicel is unicellular and short.

The secreting cell, or collection of cells, is termed a gland. Sometimes it remains unicellular, but often it divides by vertical walls or horizontal walls, or both, and thus becomes pluricellular and pluriserial (Solanaceæ, Compositæ). When division takes place by vertical walls only, it does so in a very regular manner, dividing the gland into two, four, or eight similar cells (bicellular, quadricellular, octocellular glands).

Unicellular or bicellular glands are commonly rounded or oval; they are inserted on the epidermal cells, and when they fall off leave circular scars either near the centre or close to one of the lateral walls.

Quadricellular and octocellular glands are larger and often situated in depressions on the surface of the leaf; they are usually borne upon very short pedicels, which are inserted between several epidermal cells, and consequently they leave, when they fall off, a scar differing from that left by a unicellular or bicellular gland.

All these hairs are best seen in surface preparations.

Mesophyll.—The mesophyll is said to be *homogeneous* when the cells of which it consists exhibit but little difference in shape, &c. (savin leaves), *heterogeneous* when they assume distinctly different forms. The latter is by far the more common, most leaves exhibiting a palisade tissue well differentiated from the spongy parenchyma. Two forms of heterogeneous mesophyll are distinguished—viz. *dorsiventral* and *isobilateral.* Most leaves possess a distinctly dorsiventral mesophyll, and the presence of an isobilateral structure should, therefore, be carefully noted.

The shape and size of both the palisade and the spongy parenchyma cells should be carefully observed, as well as the nature of their contents. The presence or absence of crystals, and, if present, their shape and nature, afford valuable evidence in establishing the identity of a leaf. They generally consist of calcium oxalate, and are usually rather small. Whenever they exist in a leaf they are always to be found in the powder, however fine it may be. Some leaves (foxglove) are entirely free from crystals, but most officinal leaves contain them distributed throughout the mesophyll, the cortex of the midrib, and the bast of the midrib and veins. Their shape generally remains constant in leaves of the same plant, but it may vary for different plants of the same natural order. Thus, in belladonna leaves the calcium oxalate usually assumes the form of sandy crystals, in henbane it occurs in small prisms, while in stramonium cluster crystals prevail. In senna leaves both prisms and cluster crystals are found.

The mesophyll may also contain various forms of secretory tissue, such as oil cells, oil glands, secretion ducts, laticiferous cells, laticiferous vessels, &c. The following are the principal natural orders in which these secretion cells, &c., occur:[1]

Secretion Cells.—Calycanthaceæ, Magnoliaceæ, Anonaceæ, Nymphæaceæ, Canellaceæ, Rutaceæ, Simarubeæ, Meliaceæ, Compositæ (some), Sapotaceæ, Piperaceæ, Chloranthaceæ, Myristiceæ, Monimiaceæ, Laurineæ.

Elongated Secretion Tubes, with brown contents.—Anacardiaceæ, Leguminosæ (Papilionaceæ, Mimoseæ), Compositæ, Myristiceæ, Monimiaceæ, Euphorbiaceæ.

Internal Schizogenous Glands.—Hypericineæ, Guttiferæ, Rutaceæ, Myrtaceæ, Rubiaceæ, Compositæ.

Secretion Ducts.—Hypericineæ, Pittosporeæ, Guttiferæ, Dipterocarpeæ, Burseraceæ, Anacardiaceæ, Leguminosæ (Cæsalpinieæ), Umbelliferæ, Araliaceæ, Compositæ (Cichoriaceæ).

Laticiferous Cells.—Apocynaceæ, Asclepiadeæ, Euphorbiaceæ, Urticaceæ (Humulus and Moreæ).

Sclerenchymatous cells or fibres are also occasionally present (tea, witchhazel), and then constitute important diagnostic characters (compare 'Tea').

Midrib.—The elements of which the midrib and lateral veins consist may sometimes be turned to account in identifying a leaf, although their structure presents general features common to most leaves. The section should always be made at a point distant one-third of the length of the lamina from the base ; otherwise sections from different leaves of the same species are not strictly comparable.

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[1] For complete list see Solereder, *Anatomie der Dicotyledonen.*

Next to the epidermis there is usually to be found a layer of collenchymatous cells of varying extent passing into the normal cortical parenchyma (or ground tissue) of the midrib. In powdered leaves the collenchymatous cells usually exhibit their length, and are easily distinguished by their elongated shape and thickened walls.

The cells of the cortical parenchyma, on the other hand, in surface view have thin walls, and are rounded or polygonal in herbaceous plants, but usually rectangular or elongated in the leaves of shrubs.

The wood of the midrib and lateral veins may contain vessels, tracheids, &c., the size of which is sometimes important ; the bast seldom offers valuable indications, but the presence or absence of well-developed pericyclic fibres, their shape, the extent of the thickening and lignification should be ascertained, as these characters are often very important.

General Scheme of Examination

In the complete and thorough examination of a leaf the following course of procedure may be advantageously adopted :

(1) The preparation and examination of transverse sections, including the midrib, the interneural regions, and the lateral veins.

(2) The preparation and examination of longitudinal sections of the midrib.

(3) The isolation and examination of pericyclic fibres, sclerenchymatous cells, &c., if such are present.

(4) The separation and examination of the epidermis of both surfaces.

(5) The preparation and examination of the powder.

In examining leaves the student should remember that an accurate knowledge of their structure should bear the following practical results: (1) the identification of the leaf in its entire state or at least in coarse fragments; (2) the identification of the powdered leaf. The first of these objects is generally accomplished by the examination of transverse sections and of surface preparations of the epidermises. But before examining a powdered drug it is necessary to be accurately acquainted with the structure of that drug, with the size, shape, and other characters of the various histological elements of which it is composed, for otherwise it would be impossible to say whether particular elements observed in the powder are derived from the drug in question or from some foreign source, constituting in the latter case an impurity or an adulteration. Moreover, it occasionally happens that certain characters that are conspicuous in the entire drug become obliterated during the process of pulverisation. Thus large oil glands which form a conspicuous character in some drugs are so destroyed by pulverisation as to be with difficulty found in the powder. On the other hand, it is not uncommon to find certain layers of cells tenaciously retaining their relative position in the powder and presenting certain characteristic aspects.

The examination of the powdered drug is further a useful supplement to the examination of the entire drug, as details may thereby be brought to light that would otherwise escape observation.

Examination of Powdered Drugs

The following media will be found generally useful in mounting powders for microscopical examination. They must, however, be supplemented by special methods of examination adapted for particular drugs; these methods will be described in detail as the student progresses to the study of the drugs for which they are required.

Water.—One of the principal advantages of water as a medium in which to examine powdered drugs is that it exerts very little prejudicial influence upon the cell walls or cell contents. The cell walls absorb it and resume more or less completely their original condition; the cell contents, so far as they are insoluble in water, remain unaffected, and in this respect water is much less objectionable than chloral hydrate or caustic potash. Delicate details are also more easily visible in water than in either chloral hydrate or glycerin. On the other hand, water is destitute of that power of rendering tissues transparent that is possessed both by chloral hydrate and glycerin, and hence underlying tissues that are visible in either of these media may be entirely hidden from view in water. The solvent power of chloral hydrate on many cell contents (remains of protoplasm, starch, chlorophyll, and many other substances), to which its clearing action is partly due, renders it in so far inferior to water as it exhibits the cell contents after they have been more or less altered by the action of the mounting medium.

Previous to examination under the microscope the powder should remain in contact with water for at least a few hours, in order to allow the cells and cell walls to be thoroughly penetrated.

Glycerin, pure or diluted.—Glycerin is useful, as it renders tissues more transparent than water does. It has a much less injurious influence upon the cell walls and cell contents than chloral hydrate, and, especially if used diluted, is very useful. Unlike water and chloral hydrate, it is of course not liable to evaporate or crystallise, and therefore preparations in glycerin may be kept for a considerable time under observation. The examination both in dilute glycerin and in water should not be omitted, as it frequently reveals valuable details. A good method of procedure is to mix the powder thoroughly with dilute glycerin and allow several hours for the liquid to penetrate the tissues before examining it.

Both water and glycerin allow of the determination of the colour of the fragments, and this often affords valuable information.

Solution of Chloral Hydrate.—This induces more swelling of dried and contracted cell walls than water does, a function which may, however, be disadvantageous, as very delicate cell walls may become abnormally swollen. At the same time its powerful solvent action on starch, proteid matter, resin, and other substances makes it an excellent clearing agent, and its high refractive power renders the particles more transparent than does water or glycerin. Naturally these two functions may also be disadvantageous, because important cell contents may be removed and delicate markings may be rendered invisible. But as a general mountant for powders for the exhibition of the more resistent cell walls, for expanding the cells, and rendering thicker particles more transparent, it is extremely useful. A little of the powder may be moistened with alcohol and solution of chloral hydrate added, or it may be mixed direct with solution of chloral hydrate and allowed to stand for twenty-four hours before examination. If it is impossible to allow sufficient time for this, the preparation may be gently warmed.

Alcoholic Solution of Chloral Hydrate is a better solvent of fat, wax, resin, &c., than an aqueous solution, and may in special cases advantageously follow the aqueous solution.

Solutions of other substances (e.g. sodium salicylate, zinc chloride, calcium chloride, lactic acid) are also occasionally used.

Alcohol.—Apart from its use for removing air from powders, previous to mounting them in other liquids, the chief value of alcohol is as a mountant for powders containing mucilage or other substances that swell considerably in water, and these become thereby so transparent as easily to escape observation (walls of the endosperm cells of nux vomica, foenugreek, &c.). As it induces contraction rather than expansion of the cell wall and has a low refractive index, powders mounted in it exhibit less of their structure than they do in water, glycerin, or solution of chloral hydrate, and it is therefore suited for special purposes only.

Solution of Iodine.—Iodine, as already observed, is the principal reagent for starch, and a diluted solution of iodine in potassium iodide should be regularly used as a mountant for powders in order to determine the presence or absence of that important cell content.

Solution of Potash.—This is a more powerful reagent than solution of chloral hydrate. It induces, especially if concentrated (20 to 50 per cent.), a very vigorous expansion of the cell wall, and will therefore cause tissues to resume their normal shape when solution of chloral hydrate fails. It dissolves starch and many cell contents. It is especially useful for resistent tissues and for thick, leathery leaves, particularly if they contain much colouring matter derived from tannin, but it should not be forgotten that it may induce very considerable alteration in the cell wall.

Bearberry Leaves

Source.—The leaves of *Arctostaphylos Uva Ursi,* Linn.

Transverse Sections.—Soak a few bearberry leaves in water for several hours; cut one transversely at a point distant from the base about one-third of the length of the lamina. Fix the lower part of the leaf in pith, and cut transverse sections; transfer them at once to alcohol.

Place one in a drop of water on a slide; remove the water, drop on a small drop of glycerin, and cover. If too much glycerin has been added, remove a little by means of a fragment of filter paper; take every care not to allow this (or any other mountant) to reach the upper surface of the coverslip.

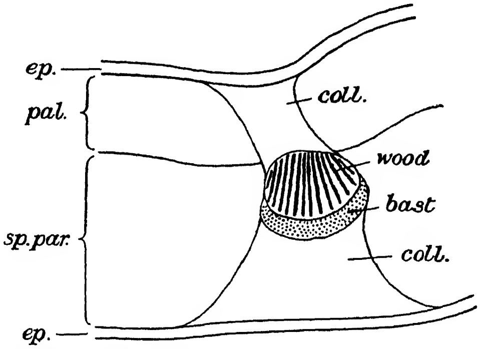

FIG. 53.—Bearberry Leaf. Diagrammatic section of midrib, showing the distribution of the various tissues. x 65.

Examine the midrib.

The wood is easily distinguished. It is fan-shaped. The elements are small but distinct; the medullary rays (high power) are radially elongated, and most of them are filled with a brown substance.

Below the wood, and extending rather more than halfway round it, is a crescent-shaped bast. The cells of this tissue are also very small, but under the high power and in favourable sections they may be distinguished.

The endodermis is not well marked, and cannot with certainty be identified.

Above and below the meristele a mass of cells with much thickened walls and angles (collenchymatous) connect the meristele with the epidermis of both surfaces. Many of these cells (especially those near the midrib) contain prismatic and cluster crystals of calcium oxalate. Their nature can be determined by radial sections, but the student is advised not to attempt this for the present. Immediately above the wood, between it and the bridge of thick-walled cells, a few sclerenchymatous fibres with very thick walls and small cavities can usually be seen (fig. 54, *bf*); they are smaller than the thick-walled cells, but larger than the elements of the wood, from which they also differ by their rounded outline and thicker walls.

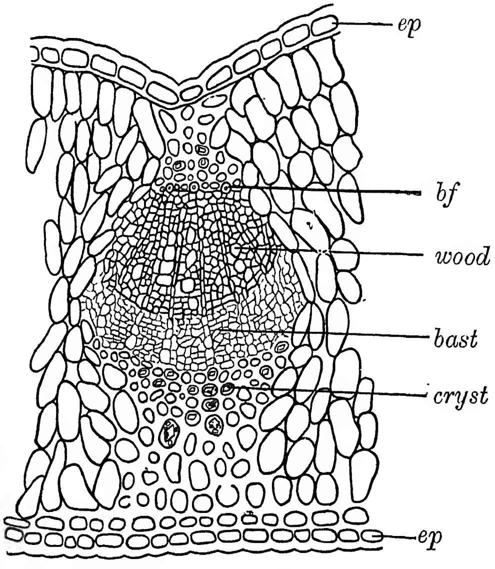

FIG. 54.—Bearberry Leaf, transverse section. × 120.

The cells of the epidermis are remarkable for their very thick cuticle.

Make a diagrammatic sketch of the section[1] (compare fig. 53).

To another section add solution of chloral hydrate, cover, and after a couple of minutes draw off the chloral hydrate and allow glycerin to take its place.

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[1] A diagrammatic sketch is intended to show the *extent* and *distribution* of the various tissues, but *not their detail.* Details of important tissues should be shown separately and on a scale large enough to enable them to be distinctly seen (compare figs. 35, 71, 88. See also p. 112).

The section clears rapidly in chloral hydrate. The crystals of calcium oxalate can now be seen very distinctly as dark masses filling the cells in which they occur. In the bast the cell walls may have swollen a little.

Examine the interneural mesophyll. It shows on the upper side several (two to four) rows of short palisade cells ; here and there an occasional crystal of calcium oxalate may be observed. The lower portion of the mesophyll consists of spongy parenchyma.

Observe the lateral veins. Like the midrib, they are connected with the epidermis by a bridge of thickened cells, and are also accompanied by fibres, which are more numerous in proportion to the wood than they are in the midrib.

Stain a section with solution of chlorzinciodine. Observe the thickness of the cuticle and cuticular layers, which stain yellow.

Surface Sections.—Next proceed to examine the surface of the epidermis. For this purpose surface sections must be prepared.

Take a well-soaked leaf, bend it over the forefinger and cut thin sections from the upper surface. Only the first section from any particular point can be utilised. Transfer these to alcohol on the slide, taking care to keep the cuticular surface uppermost. Replace the alcohol by water and the water by glycerin.

The outlines of the cells can be distinctly seen, especially near the edges of the section. They are polygonal, the walls being often slightly wavy ; there are no stomata.

Focus the epidermal cells on a thicker part of the section, and then gradually focus down until the palisade cells which lie below the epidermis of the upper surface (compare transverse section) appear distinct. Observe that the palisade cells are rounded in outline, are rather smaller than the epidermal cells, and have thin walls ; they often exhibit this aspect when surface sections or powdered leaves are examined.

Sketch a few epidermal cells together with a few of the subjacent palisade cells as they appear in a surface section (compare fig. 55, I and II).

Cut and examine in the same way sections from the under surface of a leaf.

The epidermal cells are similar to those of the upper surface, but are generally rather larger, those above the veins showing a little difference from those above the interneural regions. The latter bear stomata, often apparently arranged in groups. Examine one of the stomata more closely under the high power; it is surrounded by six or eight cells, which, however, show no definite arrangement.

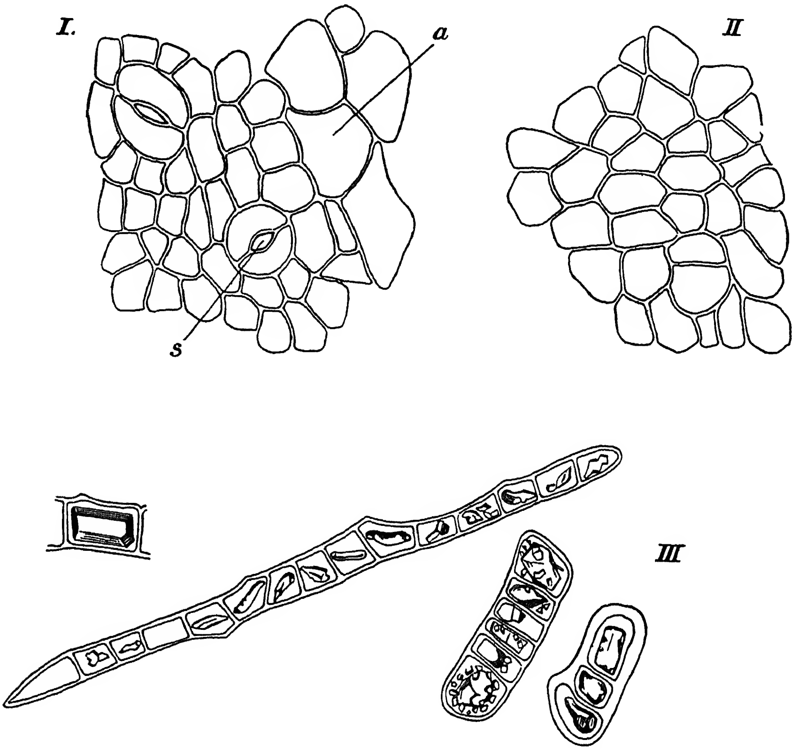

FIG. 55.—Bearberry Leaf. *I*, lower epidermis; *a*, portion over a vein; *s*, stoma; *II*, upper epidermis; *III*, cells from cortex of midrib, showing calcium oxalate (isolated by maceration with potash). ×220.

With the low power examine a larger area; hairs or glands are very seldom to be found.[1]

Separation of the Epidermis by Caustic Potash.—Macerate a few pieces of the leaf for half an hour in 2 per cent. solution of caustic potash in a water-bath; wash several times with distilled water.

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[1] In the powder, short, one-celled, thick-walled, conical hairs are occasionally to be found, as well as glandular hairs; these are derived from the margin of very young leaves.

Transfer a piece to a slide, and with the needles strip the epidermis from both under and upper surfaces; free it from adhering cells by brushing with a camel's-hair brush, and mount in water, taking care that the cuticular surface is uppermost.

The upper epidermis shows an appearance very similar to that observed in the surface section.

In the lower epidermis it will probably be observed that the cell walls, instead of being sharply defined, are indistinct and granular. This is due to the action of the caustic alkali.

Separation of the epidermis by maceration in caustic potash often yields excellent results, especially in cases in which the preparation of surface sections is difficult. The strength of the solution of potash and the length of time during which the heating should be continued vary with the nature of the leaf. Usually thin leaves require a weaker alkaline solution (0·2 to 1 per cent.) and a shorter maceration (10 to 20 minutes) than thick, leathery leaves. The foregoing comparison shows, however, that it is always desirable to ascertain, by surface sections or other preparation, whether the caustic alkali has materially altered the appearance of the cell walls. For other methods of examining the epidermis see p. 108.

Examination of Crushed Leaves.—The student should now proceed to examine coarse fragments of bearberry leaves, with a view to identifying them as such.

Coarsely crush some bearberry leaves and remove the finer fragments by sifting on a No. 20 sieve. Examine the coarser pieces as follows:

Soak them in water until sufficiently soft for cutting. Select some of the larger pieces, embed them in pith, and cut sections, taking care to cut the veins at right angles, if possible. If necessary, fix the smaller fragments on to the pith by means of a little gum and glycerin (see list of reagents), which should be allowed to dry sufficiently to hold the leaf fast.

Treat the sections as described above. Examine and sketch the transverse section of a vein and also of an interneural portion. Compare these sketches with the sketches made during the examination of the leaf itself. In the veins note particularly the bridge of thick-walled cells which connects the meristele with both upper and lower epidermis; also the sclerenchymatous fibres above the wood. In the section of the interneural portion of the lamina note the thick cuticle and the several layers of palisade.

Next digest some of the fragments in solution of potash, wash, and with the aid of needles strip the epidermis of both surfaces. Examine and sketch these, and compare the sketches with those of the leaf.

Indian Senna Leaves

Source.—The leaflets of *Cassia angustifolia*, Vahl.

Preparation for Cutting.—Select a few large and well-preserved Indian senna leaves; allow them to remain in a moist atmosphere until they are supple, but not longer, or they will become too moist. As a rule, this treatment is the most suitable for thin, papery leaves, such as senna, while soaking is preferable for thick, leathery leaves, such as bearberry. The condition that is most favourable for section cutting has been described in Section IV (Ergot).

Preparation of Transverse Sections.—Cut several leaves transversely through the midrib at the point at which the sections are to be taken (one-third of the distance from base to apex), and cut away the lamina on each side of the midrib until the width is reduced to about 3 or 4 mm. Place four or five such pieces one on the other, keeping the cut edges level, and embed the entire packet in elder-pith, so that every stroke of the razor will cut a number of sections at once, instead of one only; this method is advantageous for thin leaves. Transfer the sections, as they are cut, to alcohol.

Mounting and Examination.—Select a thin section, transfer it to a drop of water on a slide; replace the water by dilute glycerin. Examine the midrib. The wood is fan-shaped. Below and on either side of it observe delicate, small-celled bast tissue, forming nearly a semicircle. Above the wood there are a few rows of thin-walled parenchymatous cells. Both above and below there is a protecting shield of more or less thickened pericyclic fibres: this shield is crescent-shaped below the wood, but oval above it. Beyond the shield of fibres on the under surface is thin-walled parenchyma, which rapidly passes into collenchyma; on the upper side there is palisade tissue above the meristele.

Make a diagrammatic sketch of the section.

Mount another section in chloral hydrate. The cells expand and the tissue is cleared, proteid matter, &c., dissolving. The strands of bast become very distinct ; the parenchymatous cells on the outer margins of the arcs of fibres contain prismatic crystals of calcium oxalate.

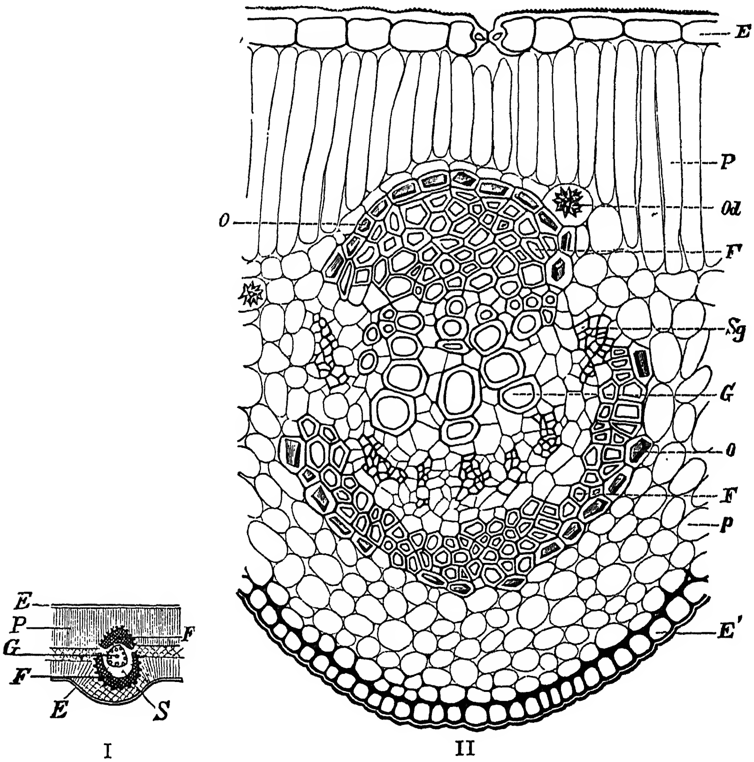


Fig. 56.—Senna Leaf, transverse sections of midrib. I. Diagrammatic ; *E*, epidermis ; *F*, pericyclic fibres ; *G*, wood ; *P*, palisade ; *S*, bast. II. Magnified 210 diameters ; *E*, epidermis of upper, *E'* of lower surface ; *F*, pericyclic fibres ; *G*, vessels ; *O*, prismatic crystals of calcium oxalate ; *Od*, rosette crystals of same ; *P*, palisade ; *p*, cortical parenchyma ; *Sg*, sieve tissue. (Meyer.)

Examine now the section of the interneural portions of the lamina. The cells of the upper and under epidermis are nearly square in outline, and covered by a distinct cuticle that bears a fine granular coating (of wax). Some of these epidermal cells appear divided into two cells by tangential walls, which are sometimes straight, but often bulge into the upper part. This appearance is due to mucilage, which is deposited on the inner tangential wall of the epidermal cell and is covered by a thin layer of cellulose; when immersed in water the mucilage swells, becomes transparent, and produces a bulging of the cellulose covering it.

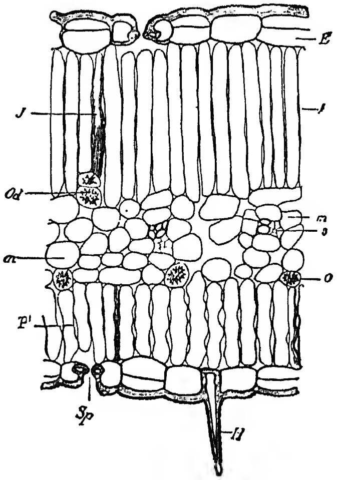


FIG. 57.—Senna Leaf, transverse section of interneural portion of lamina. *E*, epidermis; *H*, hair; *J*, palisade cell containing a strongly refractive substance; *m*, mesophyll; *p*, *p'*, palisade; *o*, *Od*, calcium oxalate; *s*, bast; *Sp*, stoma. ×210. (Meyer.)

Take a fresh section from alcohol, allow it to expand for a few seconds in chloral hydrate on the slide, wash quickly with a drop of water, and add a drop of ruthenium red solution; the mucilage in such of the cells as are intact will stain bright red (the collenchymatous tissue of the midrib and the sclerenchymatous fibres will also be stained).

Continuing the examination of the leaf section, observe the stomata which may be found on both surfaces of the leaf; the guard-cells are sunk below the level of the epidermis. Occasionally a hair may also be seen, but these are comparatively seldom found in sections. They are one-celled, thick-walled, blunt-pointed, and covered with minute warts; the base is conical, and is wedged between two epidermal cells.

Pass now to the interneural mesophyll. Note on both upper and under surfaces a layer of long narrow palisade cells (isobilateral structure), the walls of which are more (under surface) or less (upper surface) wavy. Between the palisade tissues there is a narrow layer of spongy parenchyma, the cells of which are mostly rounded; here and there a cluster crystal or occasionally a prismatic crystal of calcium oxalate can be found. Through the spongy parenchyma lateral veins and veinlets run; these possess a structure similar to that of the midrib, the elements gradually diminishing in number with the size of the veinlets until the fibres disappear and the wood is reduced to a single vessel. They are often cut obliquely, and then do not exhibit their structure clearly.

Sketch a part (about 3 or 4 cells wide) of the interneural portion of the leaf on a scale large enough to allow of the details being seen (preferably a little larger than shown in fig. 57).

Preparation and Examination of Longitudinal Sections.—Examine the structure of the midrib more closely. Prepare a leaf for longitudinal section through the midrib by cutting out a piece about 3 to 5 mm. wide, and embedding it so that the midrib is just level with the surface of the pith. Cut longitudinal sections, and transfer them all to alcohol. Mount in chloral hydrate, and examine under the low power. If there is much air, warm gently until this is expelled ; as a rule, air is more easily removed from transverse than from radial sections.

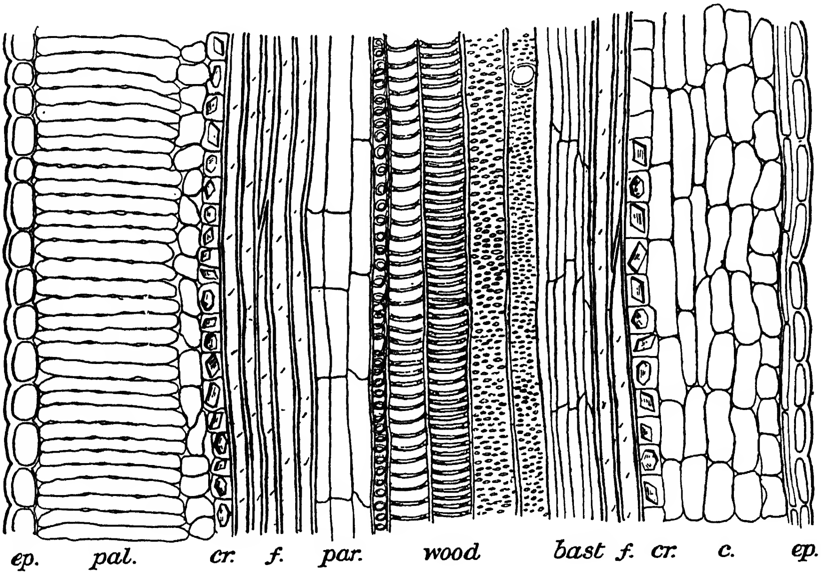


FIG. 58.—Senna Leaf, radial section of midrib. *c.*, cortex ; *cr.*, crystals of calcium oxalate ; *ep.*, epidermis ; *f.*, pericyclic fibres ; *pal.*, palisade ; *par.*, parenchymatous cells above the wood. ×180.

Select a section that has passed through the centre of the midrib—possibly several leaves will have to be cut before a satisfactory one is found—add a drop of glycerin to prevent the chloral hydrate from crystallising. Examine under the high power, and compare the tissues observed with those which the transverse section has shown to be present.

The upper epidermis can be recognised by the layer of palisade that is beneath it, as shown by the transverse section; its cells are slightly axially elongated. The palisade tissue beneath the epidermis resembles that of the interneural mesophyll, and exhibits the same irregularity in its walls. A row of cells of varying shape, but approximately isodiametric, separate the palisade cells from the crystal cells that abut upon the pericyclic fibres. If the latter are not readily distinguished, they may be made more conspicuous by appropriate staining; they are strongly elongated, rather thick-walled, and exhibit scattered slit pores arranged in a left spiral. The crystal cells are small, slightly axially elongated, and contain a single crystal of calcium oxalate in each.

Next to the pericyclic fibres are several rows of axially elongated parenchymatous cells which abut upon the vessels of the wood. The latter are spiral, annular, and pitted in succession.

The bast which follows the wood is, as a rule, but indistinctly seen. Here and there on a particularly favourable section very long narrow cells may be distinguished.

The bast is bounded by pericyclic fibres similar to those already described; next to the fibres there is a row of crystal cells.

The remaining tissue consists of the cortical parenchyma of the midrib. It is composed of axially elongated cells, which become collenchymatous near the epidermis. The cells of the latter are much smaller and more strongly axially elongated than those of the upper surface.

Further information respecting the elements of which the midrib consists may be obtained by cutting the thicker portions of the midrib from several leaves and digesting them with Schulze's maceration mixture as directed under Quassia Wood. The shape of the pericyclic fibres, vessels, &c., may be studied in this preparation.

Examination of the Epidermis.—It is frequently difficult to separate the epidermis of thin leaves by digestion with solution of potash, and recourse may then be had to the following mode of procedure:

Cut from a senna leaf several small pieces about 2 or 3 mm. in diameter; place two side by side on a slide, the one with the upper, the other with the under, surface uppermost.

Add several drops of solution of chloral hydrate, cover with a coverslip, and gently warm until the chloral hydrate just begins to boil; keep it near the boiling-point for a few moments, then cool, replace the evaporated liquid by more solution of chloral hydrate, and examine. If the operation has been successful, the leaf will have been rendered so transparent that the epidermal cells, stomata, and hairs will be distinctly visible, but some portions of the epidermis will exhibit the cells better than others, and some leaves respond better to this treatment than others; occasionally prolonged maceration in the cold affords better results than warming. If the epidermis is not clearly seen, a similar treatment with solution of caustic potash (about 0·5 to 2·0 per cent.) may be tried. The following method is also often successful: Warm two or three fragments about 2 or 3 mm. square under the coverslip in solution of potash, cool, press the finger moderately firmly on the coverslip and suddenly slide it along; by this means the epidermis may often be more or less completely detached. These various methods should be tried until a successful preparation is obtained.

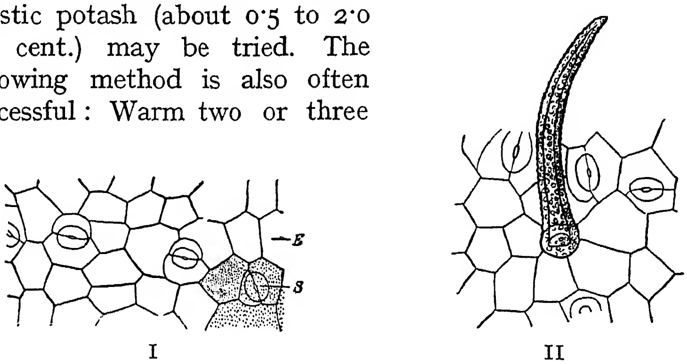


FIG. 59.—Senna Leaf. I. Epidermis of upper surface. II. Epidermis of lower surface. *E*, epidermal cell; *S*, stoma. ×210. (Meyer.)

Observe that the epidermal cells are thin-walled and polygonal in outline; stomata are found on both sides; they are usually placed between two cells, to the longer axes of which the ostiole is parallel; the latter is depressed beneath the level of the epidermis (since the microscope tube must be depressed to focus it). The hairs are very conspicuous; they are one-celled, thick-walled, and warty, and are often curved. Observe also small rounded scars which are left when the hairs fall off.

The distribution of the hairs, stomata, &c., can be well studied in fragments of the leaf made transparent in the manner described.

Depress the tube of the microscope until the palisade tissue is focussed; observe that the cells appear as small circles. Focus still farther down, below the palisade tissue, until the midrib (or a lateral vein) is focussed, and observe the regular rows of crystals; these are the calcium oxalate crystals that were seen in transverse section near the arc of fibres. They are now seen to be arranged in long axial rows.

Comparing the epidermis of the upper surface with that of the lower, observe that they are very similar; the epidermal cells have the same shape, stomata are present, and palisade tissue follows the epidermis in both cases. The lower surface, however, usually bears more hairs than the upper. Most leaves exhibit differences sufficiently marked to enable one to distinguish the epidermis of the upper from that of the lower surface; thus stomata are often absent from the upper surface, and palisade tissue is not, as a rule, to be found abutting on the lower epidermis.

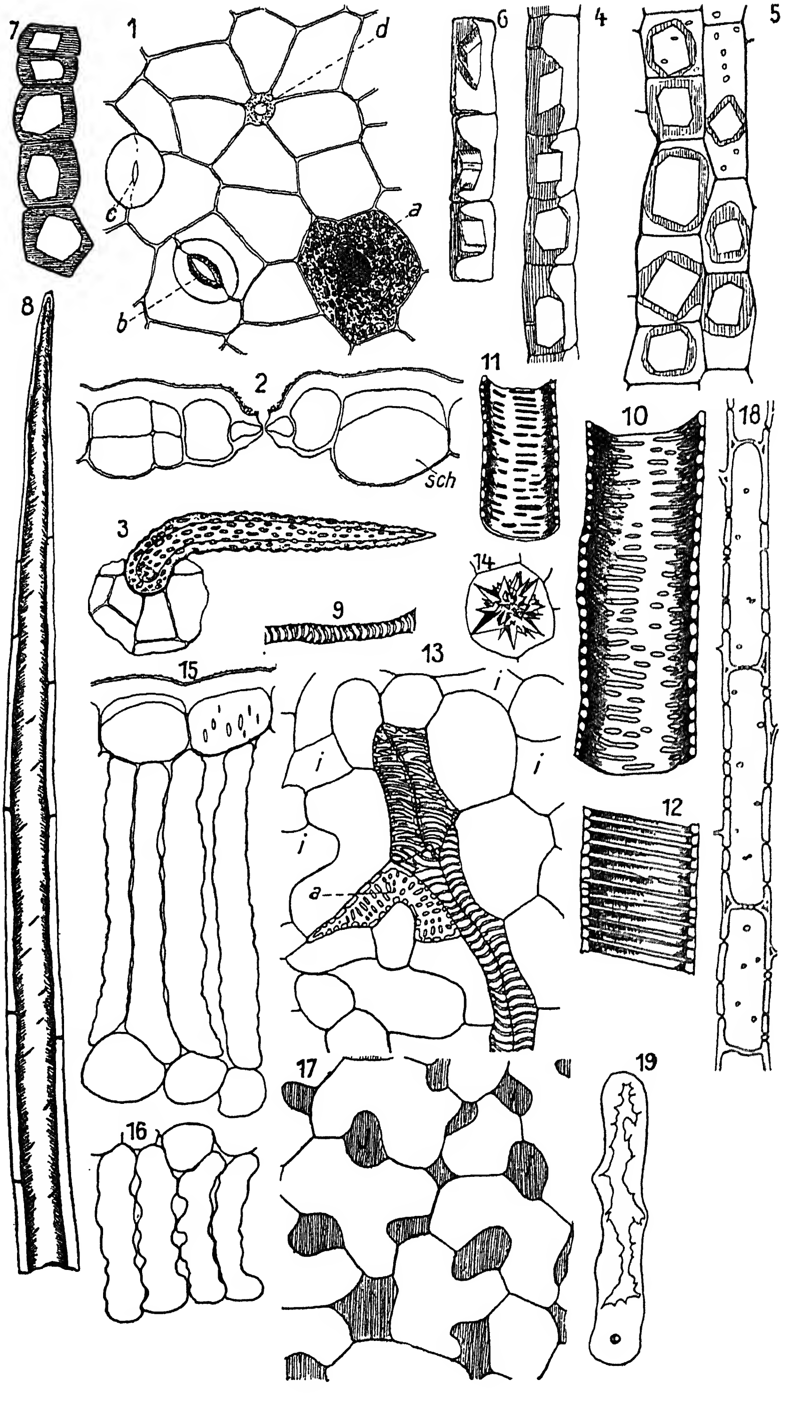
Sketching.—It is very necessary that the student should accustom himself to recording by suitable sketches the details of the tissues observed, in order that later on he may compare such details with those observed in the powdered drug. As the fragments of most powdered leaves comparatively seldom show transverse sections of the interneural mesophyll, and still more seldom transverse sections of the midrib, diagrammatic sketches of these should for the present suffice (compare figs. 53, 56, I). The *details* of the various tissues and elements should, however, be carefully drawn, care being taken to avoid all unnecessary multiplication of similar cells and to retain the correct relative size. Fig. 60 shows the structural details of a senna leaf and the manner in which they should be recorded for future reference.

Examination of Powdered Senna.—The student will now have advanced so far as to be in a position to undertake the examination of a powdered drug, and for this purpose he may select powdered senna.

It will be very undesirable for him, for the present at least, to examine any powdered drug until he has made himself acquainted with the anatomy of that drug by sections and other treatment, as directed in the preceding chapters. Indeed, in any case the most rational course to adopt is first, to determine the macroscopical characters of the drug, next the minute structure as revealed by careful, systematic investigation, and, lastly, to examine the drug in the powdered state and ascertain the changes that these tissues undergo when subjected to the operation of powdering.

A little consideration will show that the effect of pulverisation will probably be greater upon soft and delicate tissues than upon tough and hard ones, and that it will be more marked as the extent to which the pulverisation is carried increases. Sclerenchymatous fibres with comparatively thick walls may therefore be expected to resist pulverisation better than vessels, the walls of which are comparatively thin, and these again better than parenchymatous cells, the walls of which are not lignified. Moreover, those cells which are firmly held together in the drug will probably remain more or less firmly attached to one another when the drug is powdered. Such collections of cells may also present themselves to observation in positions different from those that they occupy in carefully prepared sections. Oblique views of various groups of cells will probably be met with, and be more difficult of interpretation than accurate sections, not only on account of their obliquity, but also on account of the thickness of such collections of cells and the variety of cell forms that may be associated together. Investigation shows that this is actually the case, and that, thereby, the correct interpretation of many of the particles visible when the powder is examined under the microscope is rendered a matter of some difficulty. In finely powdered leaves, for example, the cells of the palisade tissue and spongy parenchyma are often so broken up as to be scarcely recognisable. The vessels and sclerenchymatous fibres are better preserved, and can usually be identified. The epidermal cells, held together by the resistent cuticle, occur in small plates, while the collenchymatous tissue of the midribs is present in fragments, often several cells wide and thick. Hairs that are composed of large thin-walled cells suffer the same fate as the parenchymatous cells, but are more easy to identify, as they are covered by a very thin cuticle that can be stained by appropriate reagents.

In the present case, the examination of transverse and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longitudinal sections, and the investigation of the epidermis and appendages borne by it, give a sufficient knowledge of the structure of a leaf to allow of the student proceeding to the examination of the powder. This examination is, in itself, a check upon the examination of the leaf, for every cell and cell content present in the leaf must be present in the powder and, conversely, every cell and cell content in the powder must be derived from and be present in the leaf. The student should, therefore, examine the powder for the more important elements, &c., that have been observed in the leaf, and should also satisfy himself that there is no element in the powder which he cannot identify with one in the leaf.

Coarse Powder.—Dry some senna leaves in a warm drying chamber (or over quicklime) and reduce them in an iron mortar to a coarse (about No. 20 to 40) powder. Sift this coarse powder first through a No. 20 and then through a No. 60 sieve, so as to separate the coarse particles (those that remain on the No. 20 sieve) from the medium (those that remain on the No. 60 sieve) and from the fine (those that pass through the No. 60 sieve).

Examine the coarse powder first. Spread it on a sheet of paper, and examine it with a hand lens; pick out fragments with a damp brush for section-cutting. If there is any difference pick out the pieces that show it and examine them

FIG. 60. Structural Details of Senna Leaf. × 370. (Meyer.)

1. Lower epidermis, surface view; *a*, *b*, *c*, stoma with upper, middle, and lower part respectively in focus; *d*, scar of hair (in glycerin after chloral hydrate).
2. Section of epidermis with stoma; *sch*, mucilage (chloral and glycerin).
3. Hair.
4, 5, 6, 7. Vertical rows of cells with calcium oxalate crystals, from the midrib, in various positions. The calcium oxalate has been dissolved from 5, 6, and 7 by acid.
8. Fragment of a sclerenchymatous fibre of midrib, isolated by potassium chlorate and nitric acid.
9. Spiral vessel from one of the smaller veinlets.
10. Fragment of pitted vessel from midrib.
11. The same from a veinlet.
12. Fragment of spiral vessel from midrib.
13. Termination of a veinlet, from surface section. *a*, sclerenchymatous cell; *i*, intercellular space.
14. Cell containing a rosette crystal of calcium oxalate; from the powder.
15. Epidermal and palisade cells of upper surface, transverse section (glycerin after chloral).
16. Palisade cells of under surface.
17. Surface view of spongy parenchyma; *I*, intercellular spaces.
18. Collenchymatous cells from the midrib, longitudinal aspect.
19. Palisade cell, with contents of unknown nature.

separately. Take pieces of the midrib as well as of the lamina. Treat them as follows:

(1) Fix some on elder-pith previously cut and moistened with gum and glycerin; place the fragments, if possible, so as to cut the veins transversely; put them in a warm place for a few minutes until nearly dry; then adjust the other half of the pith, press together, and cut sections. Transfer the sections of pith with the sections of leaf adhering to them to alcohol, then to a slide; moisten with water, and mount in chloral hydrate, or place the pith with the sections in chloral hydrate and warm gently.

By such means sections may easily be obtained; with a little care even that which passes through a No. 20 sieve but remains on a No. 60 may be so treated.

(2) Warm some of the fragments in chloral hydrate, and examine. Should this method make the leaf transparent enough, the characters of the epidermis, &c., may be determined.

(3) Mount one or two fragments in solution of potash. Warm until the liquid boils gently, and cool. Press the coverslip firmly down and at the same time slide it along. By this means the epidermis may often be detached when chloral hydrate fails to make the leaf sufficiently transparent.

Medium Powder.—Next examine the fragments of medium size. Rub about 0·2 gramme with 10 c.c. of solution of chloral hydrate in a small mortar and transfer to a test-tube, or, better, to a centrifuge tube. Warm in a water-bath for ten to twenty minutes, remove, and allow the fragments to subside, or, far better, separate them by centrifugation. Pour off the supernatant dark-coloured liquid, and transfer a little of the deposit to a slide. Add a drop or two of solution of chloral hydrate and examine. The digestion with the chloral hydrate will have removed most of the colouring matter and made the fragments transparent.

Maceration of the powder for twenty-four hours in a closely covered watch-glass with sufficient solution of chloral hydrate to form a thin cream will also yield excellent results.

Many of the fragments exhibit the surface of the leaf, many the section; both may be compared with the sketches of senna and of the coarse powder. Numerous fragments of veins will also be found, the pericyclic fibres with their accompanying crystal cells being especially conspicuous. The details in this preparation are usually very clear.

Fine Powder.—Lastly, examine the fine powder; this will contain the majority of the small fragments of parenchymatous cells, the isolated crystals from the pericyclic fibres, sand, &c., all of which will pass through the No. 60 sieve. In addition there will be present such small fragments of the leaf as can pass through the sieve.

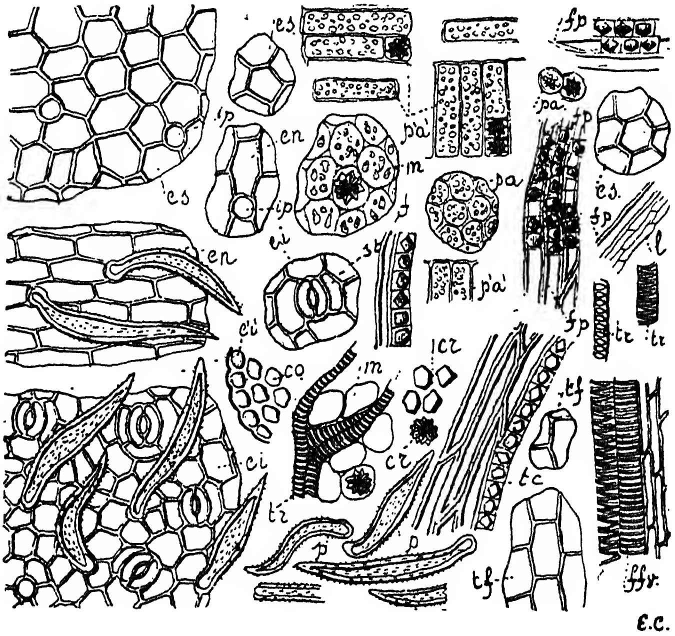


FIG. 61.—Powdered Senna. *co*, collenchymatous cells; *cr*, prismatic and cluster crystals; *ei*, *e'i'*, lower epidermis; *en*, neural epidermis; *es*, upper epidermis; *ffv*, débris of fibrovascular bundles; *f*, *fp*, lignified fibres from midrib or veins; *ip*, scar of hair; *l*, bast; *p*, hair; *pa*, *p'a'*, palisade cells; *st*, stomata; *tc*, cells with calcium oxalate; *tf*, cortical tissue of midrib; *tr*, vessels. ×240. (Greenish and Collin.)

Mix about 0·2 gramme with 10 c.c. of solution of chloral hydrate and warm for five minutes; this suffices to clear and decolourise the fine powder. Allow it to deposit, or separate it by centrifugation. Or mount a little of the powder in solution of chloral hydrate and examine after an hour.

Groups of pericyclic fibres accompanied by the crystal cells are conspicuous; isolated and broken fibres may also be observed and identified; portions of the epidermis are easily found; hairs are more or less numerous, and many isolated crystals of calcium oxalate are also to be found. Most of the delicate parenchymatous cells of the palisade and spongy parenchyma are reduced to fragments that are difficult to identify; fragments of long, narrow, cylindrical cells (palisade) with (under) or without (upper) wavy walls, rounded thin-walled parenchymatous cells with intercellular spaces (mesophyll) and rather larger elongated parenchymatous cells with thicker walls and numerous pits (cortex of midrib) may, however, be detected.

Next reduce a little of the original drug to fine powder; take about as much in bulk as a white mustard seed; moisten with alcohol, and, when nearly dry, add chloral hydrate, cover, and examine. The particles of powder will probably be sufficiently decolourised in a few minutes. Examine, and compare with the previous examination.

This preparation serves as a check upon the foregoing, and is also useful in affording information as to the relative proportion in which each of the various tissues is present.

Examination of the Fine Powder of Commerce.—Purchase some powdered Tinnevelly senna, and examine it with the object of:

(1) Identifying it as senna;

(2) Ascertaining its freedom from other leaf powders.

Mix about 0·2 to 0·5 gramme with sufficient water to make a thin cream, cover it closely and allow it to stand from 12 to 24 hours. Make similar preparations with dilute glycerin and with solution of chloral hydrate, but cover the glycerin preparation with a filter paper only (to exclude dust); then mount each in a sufficient quantity of the mountant and examine in the order named. The water and glycerin preparations will show the colour best, but the chloral hydrate preparation will be the clearest. The particles of good senna which contain chlorophyll should have fine green colour, brown or yellowish-brown particles indicating a dark, discoloured drug. This method of treatment is very useful for powdered drugs generally; care should always be taken to stir well before mounting, in order to ensure the correct proportion of the various tissues, &c., in the mounted preparation.

Mount a little in chloral hydrate, and compare with the powder of the genuine leaf; the various tissues, &c., should be present in about the same relative proportion.

Lastly, examine the powder to ascertain whether any foreign powder is present. Focus and examine every particle in the field of the microscope; it should be possible to assign to every one its position in the leaf, and impossible to find any particle differing so markedly from any of the tissues of the senna leaf as to leave little doubt of its being of foreign origin. Repeat this examination several times.

Diagnostic Characters.—(*a*) The stomata, which are bordered by two cells with their long axes parallel to the ostiole; (*b*) the one-celled, thick-walled, warty hairs, many of which are curved; (*c*) the pericyclic fibres with moderately thick walls and accompanying crystals; (*d*) the isobilateral structure; (*e*) the characteristic palisade cells of the under surface with wavy walls; (*f*) the polygonal epidermal cells on the surface of which are granules of wax.

Portions of the powder showing these characters should be sketched and the sketches compared with those made from the leaf itself.

Buchu Leaves

Source.—The leaves of *Barosma betulina*, Bart. and Wendl.

Preparation of Sections.—Prepare a leaf for cutting by exposing it to a moist atmosphere for three or four hours. Cut transverse sections of the midrib as directed on p. 99, and place them in alcohol.

Examination.—Examine one of the thinnest in glycerin. The structure is not very distinct, but the following particulars can be made out:

The outer walls of the cells of the upper epidermis are very thick, transparent, and homogeneous; the epidermal cells themselves have small cavities in which granules are visible; below the cavity of each cell there is another thick, transparent wall.

Allow water to flow on; the inner wall gradually swells and becomes invisible or nearly so; the swelling is most marked in the epidermis of the lamina between the midrib and the margin, and is so great as to lift the epidermis from the remainder of the tissue, rupturing the vertical cell walls. The swelling is due to mucilage.

Mount a section in a drop of solution of ruthenium red in lead acetate. The mucilage swells, and is coloured pink. Some of the cells of the lower epidermis also colour red, showing that they too contain mucilage. Less striking red colorations may be observed in the bast and in the walls of part of the parenchymatous tissue.

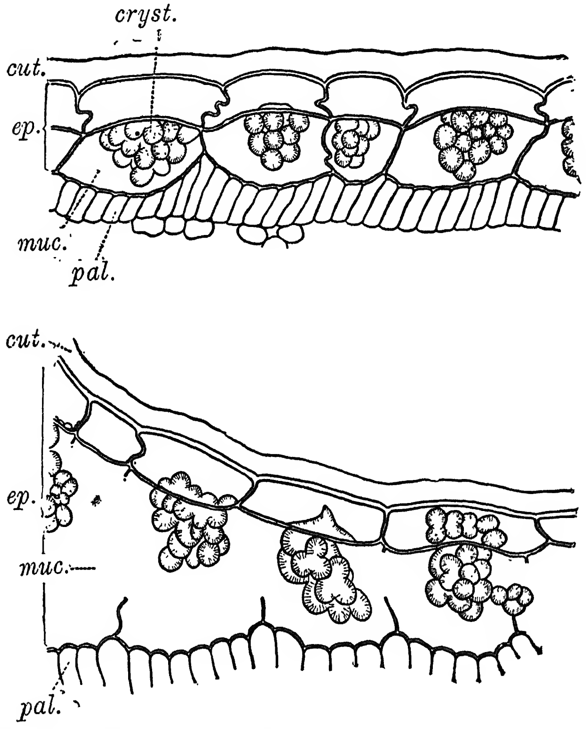


Fig. 62.—Buchu Leaf, transverse section of epidermis before and after the addition of water. *cryst.*, sphæro-crystalline masses of hesperidin; *cut.*, cuticle; *ep.*, epidermis; *muc.*, mucilage; *pal.*, palisade. × 350.

Transfer a section from alcohol to alcoholic solution of methylene blue (0·1 gramme methylene blue, 25 c.c. 95 per cent. alcohol) and after a minute or two allow a solution of methylene blue in glycerin to run on (0·2 gramme methylene blue, 10 c.c. alcohol, 40 c.c. glycerin); the mucilage is coloured blue. A saturated aqueous solution of Bismarck brown may also be used to detect mucilage, but the ruthenium red reagent is the best.

The examination of sections treated in this way would lead one to suppose that the mucilage was deposited in a layer of cells below the epidermis; this, however, has been shown not to be the case. It is deposited on the inner surface of the lower wall of the epidermal cell itself in a manner analogous to that described for senna (see p. 106).

Mount a fresh section in water; examine the granules in the epidermal cells, disregarding the mucilage; they appear crystalline. Irrigate with solution of potash; they dissolve with yellow coloration (reaction of hesperidin). Examine them more in detail from surface preparation, as described below.

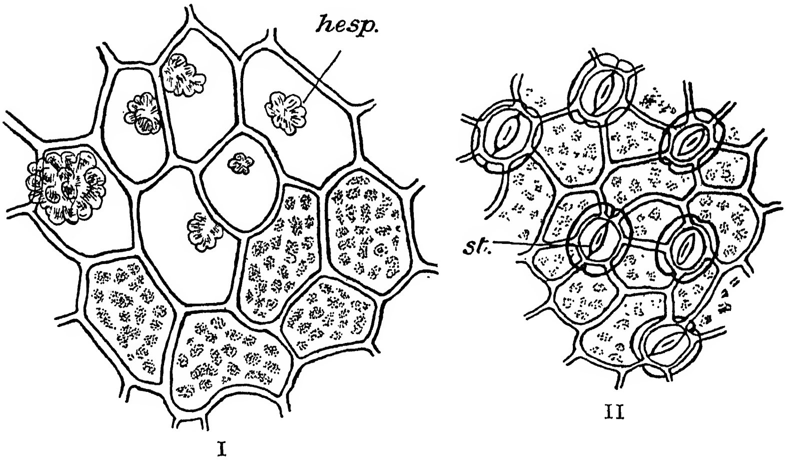


FIG. 63.—Buchu Leaf. Upper and lower epidermis. ×250.

Examine the outer wall of the epidermal cell; it appears to consist of two layers, the outer of which (cuticle) bears curious little protuberances. Irrigate with solution of chlorzinciodine. The cuticle stains yellow, the inner layer violet.

Examine the cells of the lower epidermis; they resemble those of the upper but are smaller, and scattered among them are stomata. The palisade cells contain granular matter, among which minute starch grains can be detected by the iodine reaction. In the palisade and in the spongy parenchyma there are rosette crystals of calcium oxalate, but no single crystals can be found.

Surface Preparations.—Soak a leaf in water, and when the mucilage has swelled insert a needle under the epidermis of the upper surface and strip it off. Examine in water ; if much air is entangled in the section get rid of it by one of the means alluded to on page 66. Observe first the cells : they are polygonal, and measure about 60 to 90 μ long and 40 to 60 μ wide. Their surface appears faintly and coarsely granular ; this is due to the small protuberances mentioned above. There are no stomata.

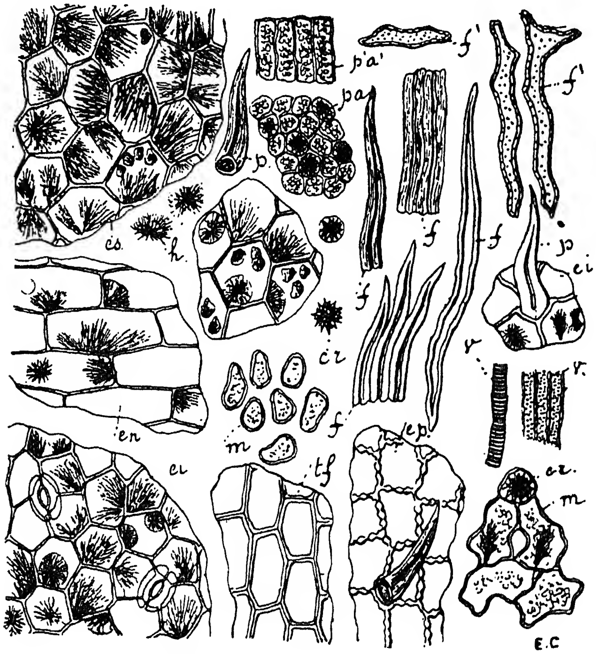


Fig. 64.—Powdered Buchu Leaves. *cr*, calcium oxalate in cluster crystals; *ei*, lower epidermis with crystals of hesperidin; *en*, neural epidermis; *ep*, epidermis of petiole; *es*, upper epidermis; *f*, sclerenchymatous fibres; *f'*, fibrous cells from veinlets; *h*, hesperidin; *m*, mesophyll cells; *p*, hairs; *pa*, *p'a'*, palisade cells; *tf*, cortical tissue of midrib; *v*, vessels. ×240. (Greenish and Collin.)

Examine the distribution of the hesperidin. Much of it is in the form of sphærites, but there are also radiating tufts as well as loose crystals. Almost every epidermal cell contains hesperidin.

To examine the epidermis of the under surface, cut thin surface sections and place in alcohol for half an hour or more. Transfer to water, and treat with solution of potash to remove the hesperidin.

The cells are polygonal, from 20 to 60 μ long, with walls that are not very thick. The stomata are very numerous, they are broadly oval, and are provided with a large ostiole. Here and there a group of a few smaller cells with thinner, pitted walls can be distinguished; these are the cells above the oil glands.

The midrib can be examined in the usual way. The wood of the meristele is fan-shaped, and the elements of which it consists are very small. Below the wood is an arc of bast, and below that again a crescent of pericyclic fibres.

Examination of the Powder.—Bearing in mind the constituents and the structure of the drug, the following method for examining the powder will prove satisfactory.

If it consists of a mixture of coarse and fine particles, separate these and examine them separately, as directed for senna. The fine powder should be treated as follows:

Mix a little with alcohol to remove air, and, when nearly dry, add

(*a*) *Solution of ruthenium red in lead acetate*; the gelatinous masses of mucilage are easily detected by the pink colour they assume: they are distributed over the whole slide.

(*b*) *Chloral hydrate*; the hesperidin can, after a few minutes, be distinguished as sphærites in the epidermal cells; it is insoluble in water, alcohol, or chloral hydrate, but soluble, with yellow coloration, in caustic potash. The cuticle appears coarsely, but faintly, granular, and is often fissured (probably due to the contraction during the drying previous to powdering).

(*c*) *Solution of potash*; the hesperidin dissolves, with yellow coloration.

The palisade cells are often in groups with the mucilage attached to them; the pericyclic fibres are in bundles, but they are free from crystals of calcium oxalate; the latter substance occurs in cluster crystals only, and is best seen in the potash preparation, as this is free from crystalline masses of hesperidin.

Tea

Source.—The leaves of *Camellia Thea*, Link.

Preparation and Examination of Sections.—Infuse some of the leaves of Congou tea in boiling water twice in order to remove as much of the colouring matter as possible. Pick out some of the larger pieces that contain the midrib as well as the margin, and remove the superfluous moisture with blotting paper. Cut from some of the fragments small portions, taking care to include the margin, and put them into solution of chloral hydrate in a small wide-mouthed bottle. They should remain a couple of days, or even longer, in this solution, and should then be used for surface preparations.

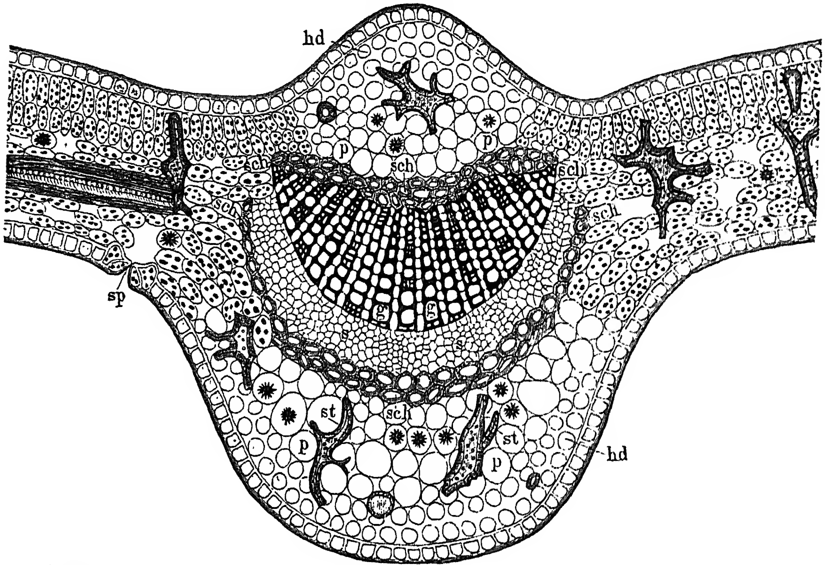


FIG. 65.—Tea, transverse section through the midrib. *hd*, hypoderma; *g*, vessels in wood; *p*, parenchyma; *s*, sieve tissue; *sch*, sclerenchymatous fibres; *sp*, stoma; *st*, sclerenchymatous idioblasts. ×130. (Warnecke.)

From other fragments cut transverse sections of the midrib as usual.

The leaf is bifacial; the epidermis of both surfaces is composed of small cells, and may bear long hairs; there are often two rows of palisade cells, but sometimes there is only one; the spongy parenchyma exhibits large air-spaces. In the centre of the leaf there are numerous cells containing calcium oxalate in varying forms, cluster crystals being often associated with small (sandy) crystals in the same cell.

In most varieties of tea there are remarkable sclerenchymatous cells (idioblasts) in the mesophyll. These cells are usually conspicuous in old leaves, or can easily be made so by staining with phloroglucin and hydrochloric acid, as their walls are strongly lignified. They may occur in the lamina as well as in the cortex of the midrib, but in young leaves (such as constitute Pekoe tea) they are found in the midrib only and are thin-walled, although also lignified; whereas, in the bud, they may not even be lignified. If any difficulty be experienced in finding them, cut from the under surface of the leaf tangential sections of the midrib; some of these will pass through the cortex, and exhibit the sclerenchymatous cells well.

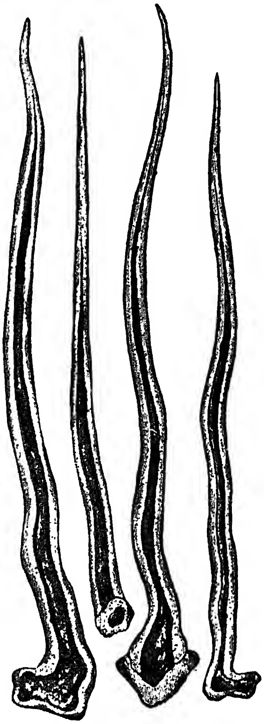


FIG. 66.—Hairs of Tea Leaf. (Moeller.)

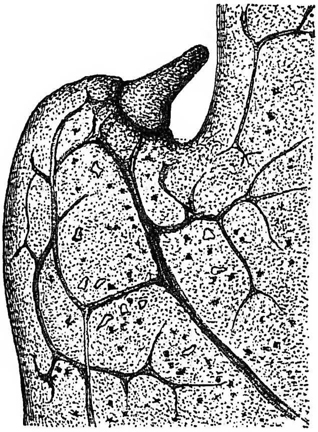


FIG. 67.—Tea Leaf, cleared by chloral hydrate, showing the veinlets, marginal tooth, and distribution of sclerenchymatous idioblasts, and calcium oxalate crystals. Slightly magnified. (Schimper.)

Surface Preparation.—Examine next the leaf that has been soaked in solution of chloral hydrate, taking the under surface first. Observe the hairs. These are less abundant on old leaves than on young ones. They are often very long, many attaining 500 to 700 μ in length ; they are narrow, one-celled, and thick-walled ; they are bent nearly at right angles near the base, so as to lie almost flat on the surface of the leaf : they are often surrounded at the point of insertion by radially arranged epidermal cells.

Next examine the epidermis. The cells are not very easy to see and require careful adjustment of the light ; if difficulty is experienced, cut surface sections, soak them first in chloral hydrate, then in glycerin, and finally transfer to water, or transfer them from chloral hydrate to a more dilute solution of the same reagent.

The cells are slightly wavy in outline, distinctly so in old leaves, scarcely perceptibly so in young ones; they often exhibit traces of the treatment the leaves have undergone. The stomata are broadly oval, and exhibit a narrow ostiole ; they are surrounded by three or four narrow, tangentially arranged cells. These stomata are characteristic, and should be carefully observed.

Lastly, examine the venation of the leaf, and the teeth at the margin. Each tooth is a conical mass of parenchymatous cells, and often falls off, leaving, in old leaves, a brown scar. A veinlet runs up to this scar, and, there, generally spreads a little. The teeth and venation are characteristic.

The epidermis of the upper surface consists of small, delicate, polygonal cells, and exhibits no stomata.

Young leaves (Pekoe tea) are thinner and greener, and are more easy to clear and examine than the older and darker leaves of which Congou tea consists. The sclerenchymatous idioblasts are to be found in the midrib only, and the teeth are usually still attached.

The following characters serve to identify the leaf :

Diagnostic Characters.—

(*a*) The hairs, their shape and size, together with the radiate arrangement of the cells at the base.

(*b*) The crystals of calcium oxalate ; they are cluster crystals, and are often accompanied by crystal sand.

(*c*) The sclerenchymatous idioblasts, especially those of the petiole and midrib ; they are never entirely absent.

(*d*) The stomata.

(*e*) The teeth at the margin, or the scars left by them.

Examination of the Powder.—Powdered tea may be examined as directed for powdered senna. Digestion with chloral hydrate and subsequent separation by centrifugation, repeated a second time, is strongly to be recommended. The final deposit can be washed free from chloral hydrate (separating by the centrifuge) and stained with appropriate reagents (e.g. phloroglucin and hydrochloric acid, by which the sclerenchymatous cells are at once made conspicuous).

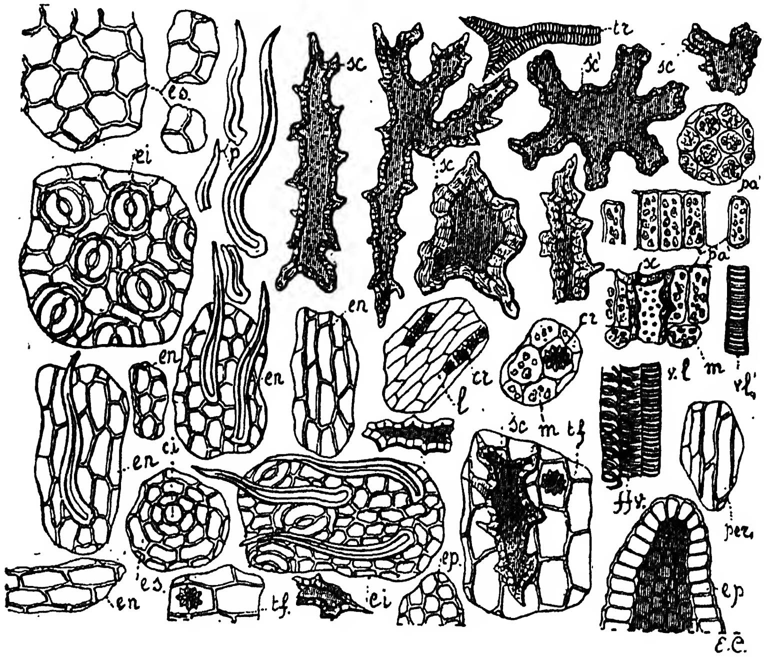


FIG. 68.—Powdered Tea. *cr*, crystals; *ei*, lower epidermis; *en*, neural epidermis; *ep*, apex of marginal tooth; *es*, upper epidermis; *ffu*, débris of fibrovascular bundles; *l*, bast with cluster crystals; *m*, spongy parenchyma; *p*, simple hairs; *pa*, *p'a'*, palisade cells; *per*, pericycle, slightly lignified; *sc*, idioblasts from the mesophyll and cortical tissue; *s'c'*, idioblasts from the pith of the stem; *tf*, cortical tissue; *tr*, tracheids; *vl*, vessel. ×240. (Greenish and Collin.)

Powdered tea may also be examined by moistening a little on a slide with alcohol, allowing it nearly to dry, adding chloral hydrate, and gently warming.

Stramonium Leaves

Source.—The leaves of *Datura Stramonium,* Linn.

Preparation and Examination of Sections.—Soften in the usual way. Cut and examine sections of the midrib, with part of the adjoining interneural portions.

The midrib is convex above and strongly convex below. The wood of the meristele has the shape of a rather flat arc; it contains large vessels, often 30 μ or more in diameter, and is provided with bast both above and below (a constant character in plants belonging to the order Solanaceæ). The bast contains no sclerenchymatous elements, nor are there any pericyclic fibres.

The endodermis cannot be distinguished. The cortex is composed of large cells, some of which are filled with sandy crystals of calcium oxalate; cluster crystals and prisms may also be found, but they are less frequent.

The interneural spaces exhibit an asymmetrical, heterogeneous mesophyll. The palisade cells are long and narrow, and occupy about one-half of the mesophyll. The spongy parenchyma is rather dense. Cluster crystals of calcium oxalate are present in abundance, they are situated chiefly in the spongy parenchyma abutting on the palisade; some prismatic crystals and occasionally a cell with sandy crystals may also be found.

Surface Preparations.—Warm some fragments of the lamina (between the stronger veins) in chloral hydrate on the water-bath, for about half an hour. Examine in chloral hydrate. The features of the epidermis and the distribution of the stomata can generally be easily seen. This method, however, occasionally fails, in which case one of the others described on pp. 101 and 109 should be tried.

The epidermal cells vary considerably in size as well as in outline. For the same leaf the lower epidermis has cells with more wavy walls than the upper. Stomata are to be found on both surfaces; they are surrounded by three or four cells, one of which is smaller than the others, a common feature in solanaceous leaves.

Two forms of hairs are present, simple and glandular; they are generally more numerous on young leaves and near the veins. On older leaves there are often but few to be found.

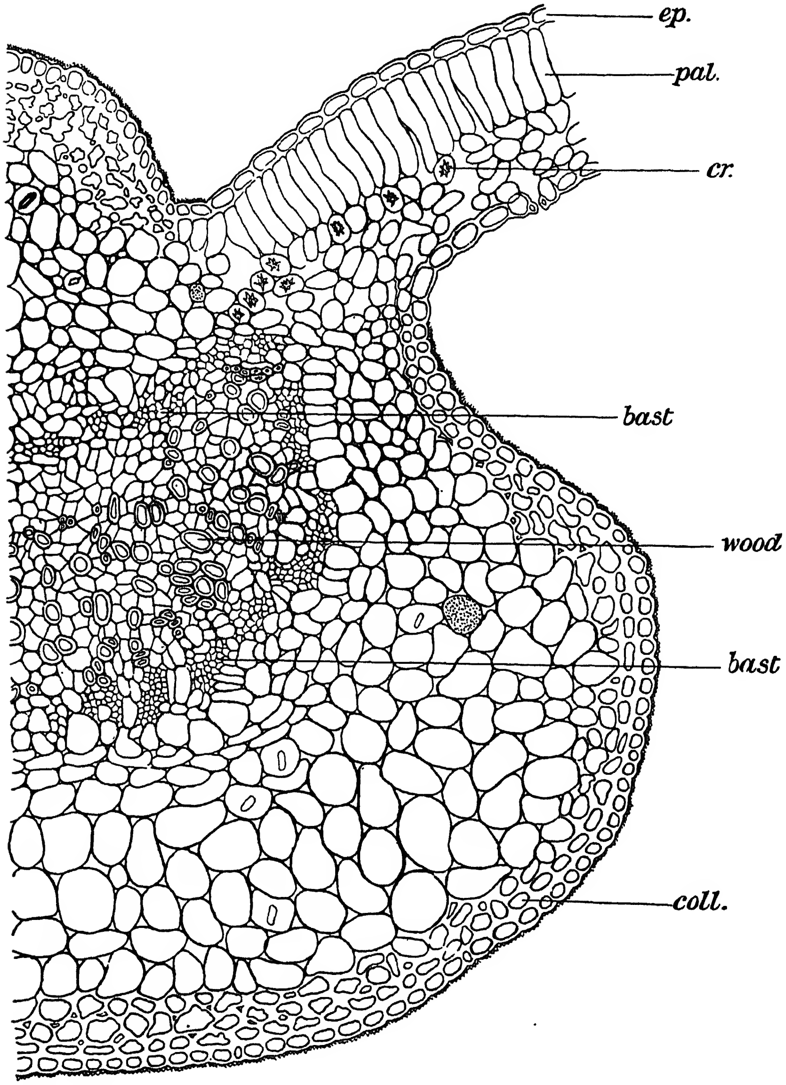


FIG. 69.—Stramonium Leaf, transverse section through the midrib. *ep.*, epidermis; *cr.*, cluster crystal of calcium oxalate; *coll.*, collenchymatous tissue; *pal.*, palisade. (After Tschirch.)

The simple hairs are uniserial and conical. They are composed of from three to five cells, the walls of which are warty and not very thick.

The glandular hairs are short, and consist of an oval pluricellular gland supported upon a pedicel.

Examination of the Powder.—Powdered stramonium is best

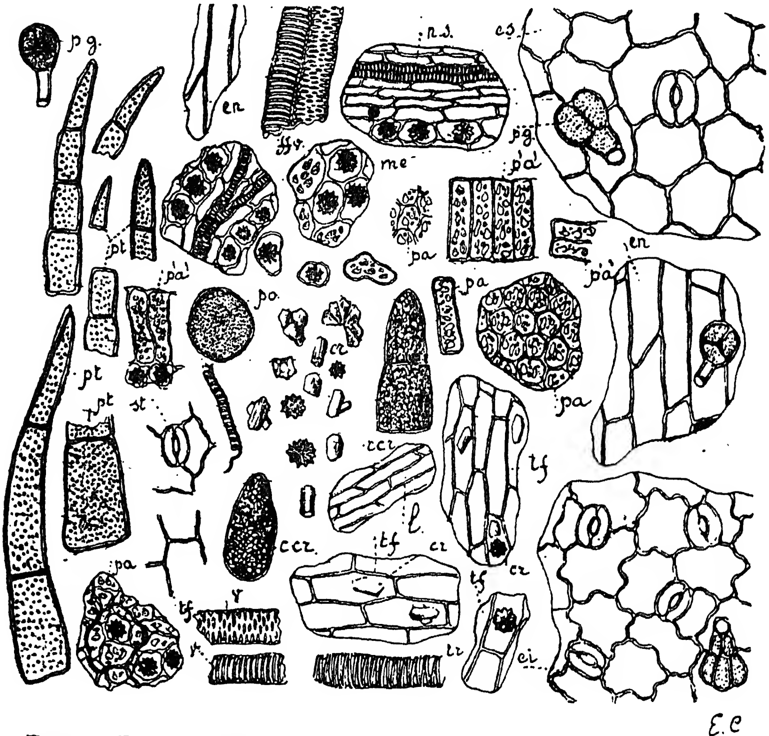


FIG. 70.—Powdered Stramonium Leaves. *cr*, crystals; *ccr*, crystal cells; *ei*, lower epidermis; *en*, neural epidermis; *es*, upper epidermis; *ffv*, débris of fibrovascular bundles; *l*, bast; *me*, spongy parenchyma; *pa*, *p'a'*, palisade tissue; *pg*, glandular hairs; *po*, pollen grains; *pt*, simple hairs; *tf*, cortical tissue of midrib; *tr*, *v*, vessels, &c. ×240. (Greenish and Collin.)

prepared for examination by the method described under Powdered Senna (pp. 113 to 117). The powder is liable to contain much sand; this is conspicuous in the deposit obtained by allowing the mixture of powdered stramonium and chloral hydrate to stand for a few moments. The suspended particles should then be separated and examined.

Some fragments exhibit the surface of the leaf, but there are many that exhibit the section; and these can easily be identified by comparison with the sketches previously made, noting particularly the form and distribution of the calcium oxalate. The epidermis is, as a rule, not well seen even in those fragments that present their surface to the observer. Groups of axially elongated cells derived from the cortical parenchyma of the midrib are frequently to be found, the cells of which they consist being often rather large. Cluster crystals of calcium oxalate are abundant, but pericyclic fibres are not to be detected. The vessels are often of considerable size.

Among the smaller fragments, portions of the epidermis and of the cortex of the midrib may be found. In addition to these fragments of tissues which are readily identified, there are abundant *débris* of cells, portions of cell walls, &c., the origin of which is not so easily ascertained. The hairs, characteristic when present, are often so rare as to require careful search; they are usually more or less broken up, so that fragments only can be found (for a means of staining these see p. 138).

Mount also fresh preparations of the powdered leaf in chloral and in dilute glycerin, allowing them to stand for a short time before examining them.

Diagnostic Characters.—Diagnostic characters are to be found in

(*a*) the characters of the transverse section (which may always be found), especially the long, narrow, palisade cells and the calcium oxalate;

(*b*) the characters of the surface preparations; (especially the calcium oxalate and the epidermis when visible);

(*c*) the hairs, which are often very rare;

(*d*) the size of the vessels of the wood and of the cortical cells of the midrib.

Coca Leaves

Source.—The leaves of *Erythroxylon Coca,* Lam.

Preparation and Examination of Sections.—Select for examination well-preserved Bolivian coca leaves. Prepare them for cutting by exposing them to moist air. Cut sections of the midrib at the proper point (see Bearberry Leaves), and treat them as directed for bearberry leaves.

The midrib exhibits no remarkable features; there is usually an arc or an interrupted ring of sclerenchymatous fibres present. Above the midrib, the ridge characteristic of Bolivian coca may be seen.

The epidermis of the upper surface calls for no remark. In the palisade tissue observe occasional small prismatic crystals of calcium oxalate; in the spongy parenchyma, cluster crystals of the same salt occur.

The cells of the lower epidermis are very remarkable and should be carefully examined; they are distinctly papillose, and at the apex of each papilla the cell wall is lenticularly thickened. Sometimes the cells contain mucilage (see Senna Leaves).

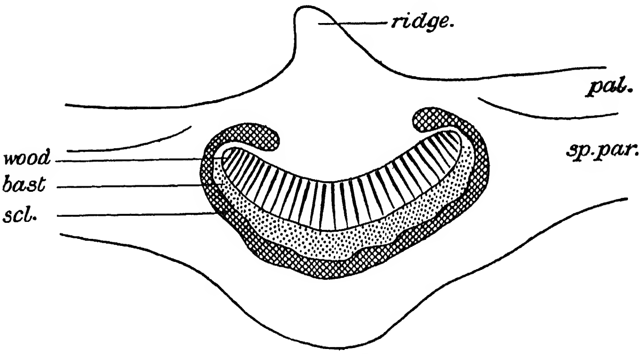


FIG. 71.—Bolivian Coca Leaf, diagrammatic section, showing the distribution of the tissues. ×65.

Prepare also transverse sections through the line that runs near the midrib from base to apex. The line is seen to be a ridge caused by the formation of several (usually five or six) smaller, collenchymatous cells beneath the epidermis; their function is probably a mechanical one.

Surface Preparations.—Examine the epidermis as directed for bearberry leaves; observe minute granules and little rods on the cuticle (wax), and in each cell, except those surrounding the stomata, a clear disc surrounded by such granules; these discs are produced by the papillæ when viewed from above (see fig. 73). Note also that the stomata are bordered by four cells, two of which have their long axes parallel to the ostiole.

Treat the leaf by warming with chloral as directed for senna; the circles on the epidermal cells are visible, but the granules have fused to minute globules ; find and examine the curved line ; note the axial elongation of the cells.

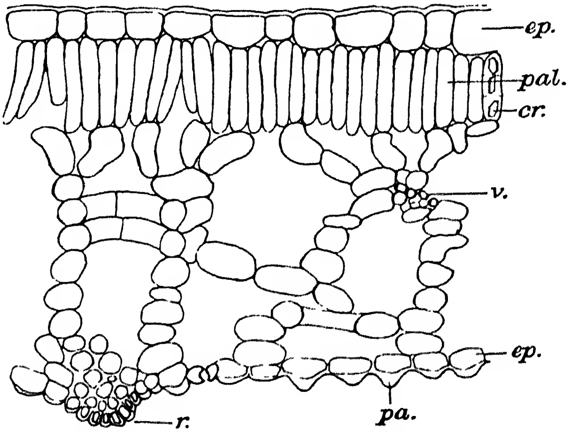


FIG. 72.—Bolivian Coca Leaf, transverse section. *cr.*, crystals of calcium oxalate ; *ep.*, epidermis ; *pa.*, papillose cells of lower epidermis ; *pal.*, palisade ; *v.*, veinlet ; *r.*, line on under surface. × 200.

Some coca leaves also contain remarkable branched sclerenchymatous cells arranged parallel to the epidermis and closely applied to it. They are easily detected in surface preparations, but may escape notice in sections. They are probably mechanical in function.

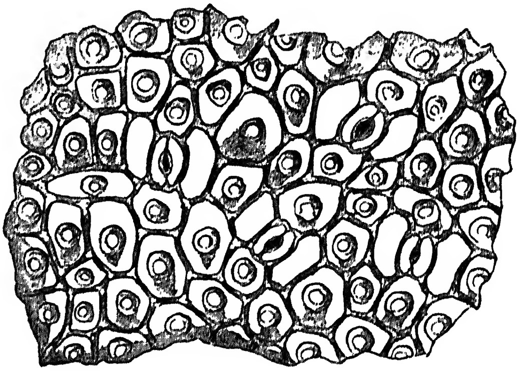


FIG. 73.—Coca Leaf. Lower epidermis. (Moeller.)

Examination of the Powder.—Examine this as directed for senna leaves.

Diagnostic Characters.—

(*a*) The small, polygonal, papillose cells of the lower epidermis.

(*b*) The small stomata accompanied by two cells with their long axes parallel to the ostiole.

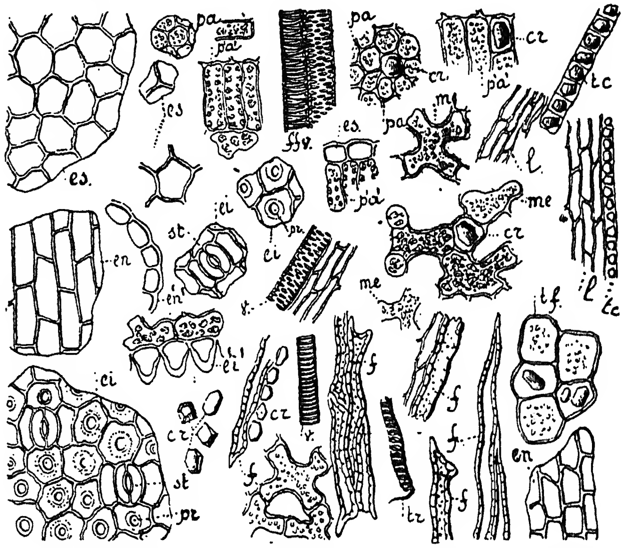


Fig. 74.—Powdered Coca Leaves. *cr*, prismatic crystals of calcium oxalate; *ei*, lower epidermis, with surface view of papillose cells (*pr*); *e'i'*, lower epidermis in section; *f*, sclerenchymatous fibres; *fv*, fragments of vessels from midrib; *l*, bast; *me*, spongy parenchyma; *pa*, *p'a'*, palisade cells; *st*, stomata, with two subsidiary cells parallel to the ostiole; *tc*, crystal cells; *tf*, cortical tissue of midrib; *tr*, vessels, &c. ×240. (Greenish and Collin.)

(*c*) The pericyclic fibres.

(*d*) The prismatic crystals of calcium oxalate.

(*e*) The absence of hairs.

Savin

Source.—The extreme tops of the twigs of *Juniperus Sabina*, Linn.

Preparation and Examination of Sections.—Soak some small twigs of savin, preferably those with small appressed leaves, for twelve hours in water. Cut transverse sections, and treat as usual.

The leaves are opposite and decussate, and the lower portion is usually adnate to the stem, the upper portion being free. On the under surface of each leaf a large oil gland can be seen. The sections cut may therefore be sections of the leaves without the stem, or sections of the small stem to which the two opposite leaves are attached.

The structure of the leaf is centric and the shape of the section nearly a semicircle. The meristele is embedded in the centre of a homogeneous mass of parenchymatous tissue; it consists of a small, slightly arched group of tracheids with bast on their lower surface. The endodermis is not distinguishable, nor is there any fibrous pericycle, but on each side of the wood there is a small group of lignified cells with irregular thickenings (transfusion tracheids); these are very conspicuous when stained with phloroglucin and hydrochloric acid.

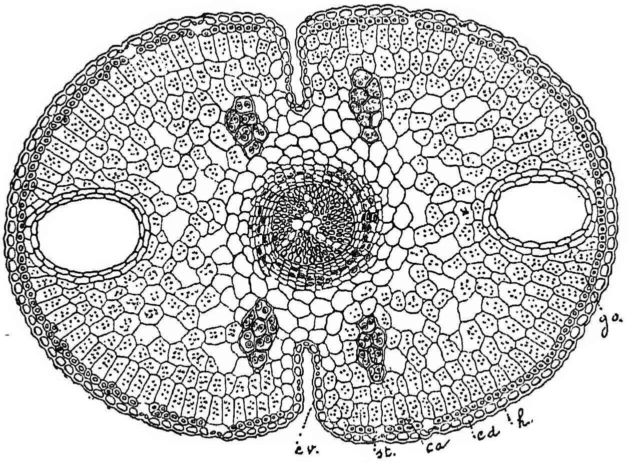


FIG. 75.—Savin, transverse section of twig. *ca*, cells with bordered pits; *ed*, epidermis of lower surface of adnate leaf; *ev*, epidermis of upper surface; *go*, oil gland; *h*, hypoderma; *st*, stoma. (Collin.)

The epidermis consists of cells nearly square in section and provided with a thick cuticle. Stomata occur on both surfaces, but on the portion of the leaf that is free, they are generally restricted to the upper surface.

Below the epidermis there is a hypoderma consisting of a single or sometimes double row of cells with small rounded outline and thickened walls. Embedded in the lower portion of the mesophyll is a large oil gland.

Stain a section with phloroglucin; observe that the wood and transfusion tracheids are strongly lignified, the hypoderma less strongly so. Examine minutely the stomata; these also yield the lignin reaction.

Maceration Preparation.—Digest a twig in solution of potash in a water-bath for fifteen minutes; wash in distilled water and tease out on the slide.

The epidermis is composed of cells with moderately thickened pitted walls. Near the stomata they are more or less isodiametric, but in other portions axially elongated.

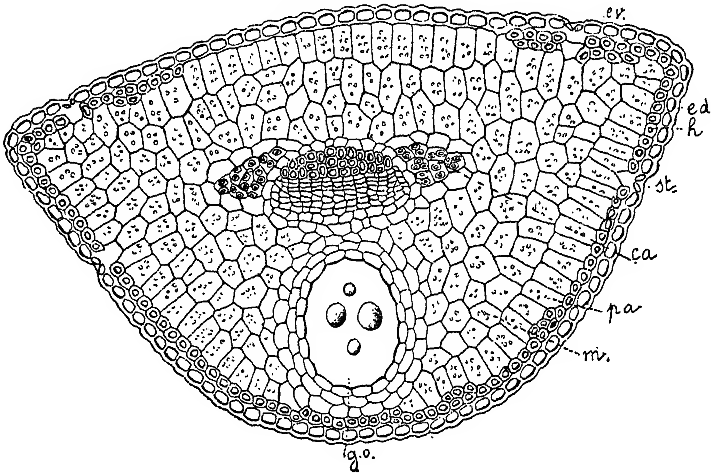


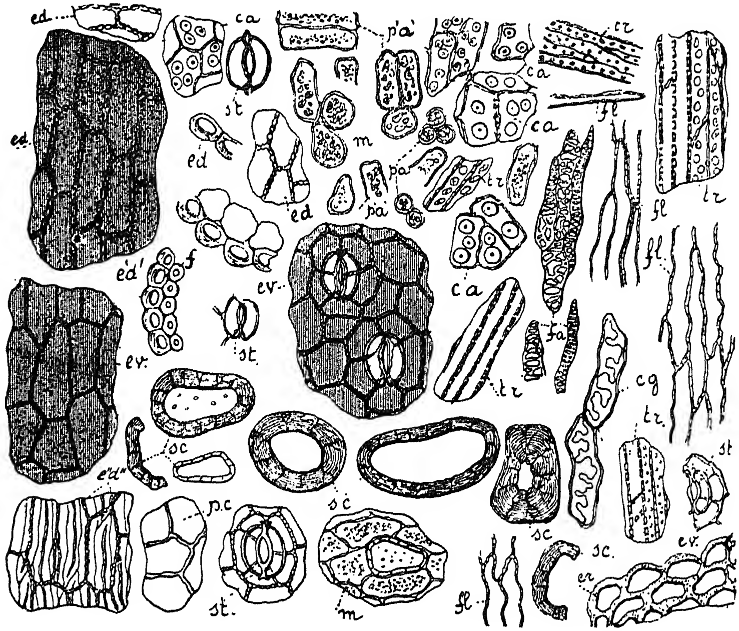
FIG. 76.—Savin, transverse section of leaf. *ca*, cells with bordered pits; *ed*, epidermis of lower surface; *ev*, epidermis of upper surface; *go*, oil gland; *h*, hypoderma; *m*, mesophyll; *pa*, palisade; *st*, stoma. (Collin.)

Below the epidermis and closely adherent to it is the hypoderma; the cells can be teased apart, and are then seen to be long and narrow, with small lumen, blunt ends, and irregular outline.

Examine the stomata carefully. They are surrounded by four or five cells, which partly overhang the guard-cells. The latter are lignified, especially on their mutually apposed surfaces, and from the extremity of the stoma there appears a small lignified projection (compare fig. 77); this appearance is very characteristic.

In the teased leaf groups of the transfusion tracheids are easily found.

Examination of the Powder.—Powdered savin is best prepared for examination by exhausting with chloral hydrate. The stomata and transfusion tracheids are very characteristic, and can be detected at once in a stained preparation. The epidermis is also easily found and characteristic, but the details of the fibrous hypoderma are difficult to distinguish, as the latter adheres pertinaciously to the epidermis. The stomata, the transfusion tracheids, the epidermis and the fibrous hypoderma render this leaf one of the easiest to identify.



Ee

FIG. 77.—Powdered Savin. *ca*, *cg*, cells with areolated pits and supports; *ed*, *e'd'*, lower epidermis; *e''d''*, the same with fibrous hypoderma attached; *ev*, upper epidermis; *fa*, reticulated fibrous cells; *st*, stomata; *tr*, tracheids; *m*, mesophyll; *sc*, sclerenchymatous cells (found only in *J. phoenicea*, the twigs of which are sometimes substituted for those of *J. Sabina*); *fl*, bast fibres from stem. ×240. (Greenish and Collin.)

Diagnostic Characters.—

(*a*) The characteristic stomata.

(*b*) The epidermis with fibrous hypoderma (often not readily visible).

(*c*) The lignified cells with areolated pits.

(*d*) The reticulated fibrous cells.

Foxglove Leaves

Source.—The leaves of *Digitalis purpurea*, Linn.

Preparation and Examination of Sections.—For examination, select preferably foxglove leaves that are not very hairy on the under surface; by this means the examination of the epidermis in surface preparations is much facilitated. Prepare them for cutting by exposing them to a moist atmosphere for twelve hours; at the same time soak some small fragments of the interneural lamina in chloral hydrate.

Cut and examine transverse sections of the midrib as usual. Observe that the wood is crescent-shaped, and possesses numerous large vessels. Below it is the bast, which is supported by a sheath of collenchymatous cells; a similar band of collenchyma is visible above. The cortex is composed of parenchymatous cells which, in transverse section, are rounded, but in radial section are (as is usual) axially elongated.

Examine the interneural spaces. The leaf is dorsiventral; the palisade cells are short and broad, and the mesophyll occupies about the same space as the palisade. The epidermal cells of the upper surface are comparatively large, those of the lower are small; the latter surface exhibits stomata that are raised above the level of the epidermis. Both surfaces bear characteristic hairs. These are either simple (protective) or glandular. The former are uniserial and pluricellular; they usually consist of three to five (exceptionally as many as ten) thin-walled cells, and are either slightly warty or nearly smooth; frequently the cells are collapsed. The glandular hairs consist of a short pedicel supporting a one- or two-celled gland. The hairs and the stomata can be better examined in surface preparations.

In no part of the section can calcium oxalate be detected.

Surface Preparations.—The surface of the leaf can best be examined by warming in chloral hydrate in a water-bath for ten to fifteen minutes, or by allowing it to stand cold for twelve to twenty-four hours; occasionally dilute solution of potash gives the best results. Cut out from a piece of a leaf all the larger veinlets until the fragments are only one or two millimetres in diameter.

The upper epidermis is usually very distinct, and is composed of polygonal cells with no stomata at all or only very few. The presence of the numerous hairs on the under surface rather obscures the field, but under tolerably favourable conditions there is little difficulty in observing the wavy outline of the epidermal cells and in detecting stomata. The hairs may also be examined in this preparation.

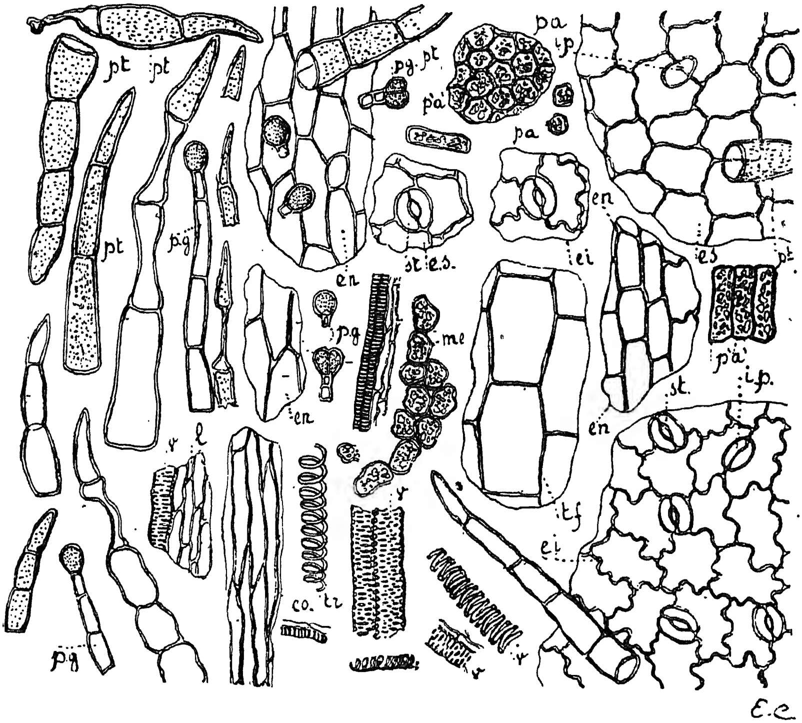


FIG. 78.—Powdered Foxglove Leaves. *co*, collenchymatous cells of the midrib; *ei*, lower epidermis, cells with sinuous walls; *en*, neural epidermis; *es*, upper epidermis; *ip*, scar of fallen hair; *l*, bast; *me*, spongy parenchyma; *pa*, *p'a'*, palisade cells; *pg*, glandular hairs; *pt*, simple hairs; *st*, stomata; *tf*, cortical tissue of midrib; *tr*, *v*, vessels, &c. × 240. (Greenish and Collin.)

Examination of Foxglove Powder.—Treat the powder as directed for senna (p. 110).

Conspicuous in the preparations are pieces of the cortex of the midrib and of the stronger veins; these are easily identified if they still have the epidermis adhering, as this will exhibit the broken bases or the scars of hairs. Fragments of the smaller veins are also very numerous; these generally have the epidermis attached and can thus be recognised; they are also seen to be free from pericyclic fibres and from calcium oxalate. Some of these fragments include portions of the interneural mesophyll, which is likewise free from calcium oxalate. Pieces are also to be found showing their transverse section, but these are not so numerous.

Among the smaller fragments of cells are numerous portions of the broken hairs. These may be more readily detected when stained as follows:

Transfer a little of the deposit (obtained by exhausting with solution of chloral hydrate) to a slide, carefully remove the chloral hydrate, wash with a drop or two of water (which also remove), and add two or three drops of Soudan red in glycerin; cover and gently boil for a few seconds, cool, and, if necessary, add a drop of glycerin. Allow the preparation to stand for a few minutes before examining it. The cuticle and the hairs will stain red, the other cell walls acquiring at most only a slight colour. The fragments of hairs can thus be found without difficulty; most of them are warty, but some are smooth.

Diagnostic Characters.—

(*a*) The wavy epidermal cells with small stomata.
(*b*) The simple hairs, mostly three- to five-celled, with thin, often warty walls.
(*c*) The absence of pericyclic fibres.
(*d*) The absence of calcium oxalate.

Belladonna Leaves

Source.—The leaves of *Atropa Belladonna*, Linn.

Preparation and Examination of Sections.—Prepare and examine belladonna leaves as directed for foxglove. Observe that the meristele contains bicollateral bundles. The mesophyll is characterised by the presence of large cells packed with minute (sandy) crystals of calcium oxalate; under the low power this appears as a dark granular substance.

The epidermis may be examined either as directed for foxglove, or it may be separated by warming for fifteen minutes in a water-bath with solution of potash (about 1 per cent.); both methods give good results, but the former is perhaps simpler.

Note the large epidermal cells with wavy walls and striated cuticle; the stomata are also large, and are situated principally on the under surface, each of them being surrounded by three or four cells, one of which is smaller than the others; this arrangement is commonly met with in solanaceous plants.

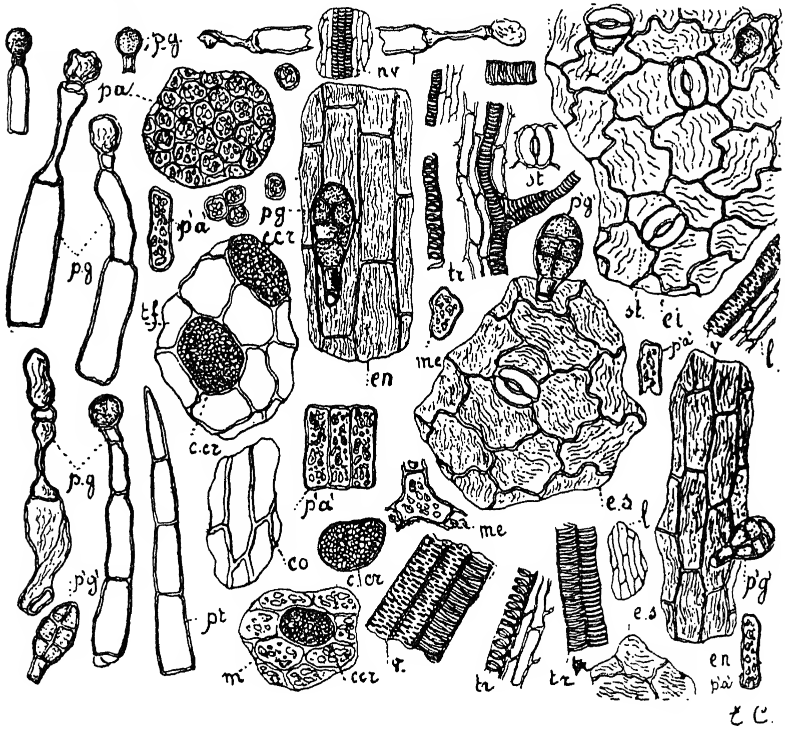


FIG. 79.—Powdered Belladonna Leaves. *ccr*, cells with sandy crystals of calcium oxalate; *co*, collenchymatous cells from cortical tissue of midrib; *ei*, epidermis of under surface; *en*, epidermis over the veins, with striated cuticle; *es*, epidermis of upper surface, with striated cuticle and occasional stomata; *l*, bast; *me*, branching cells of spongy parenchyma; *nv*, fragments of small vein; *pa*, *p'a'*, palisade cells; *pg*, *p'g'*, glandular hairs, long and short, with unicellular and pluricellular glands; *pt*, simple hairs; *st*, stomata surrounded by three or four cells, one of which is smaller than the others; *tf*, cortical tissue of midrib; *tr*, *v*, vessels, &c. ×240. (Greenish and Collin.)

Hairs may occasionally be found. They are of two kinds, simple or glandular. The former are uniserial, and consist of two, three, or four thin-walled cells. The latter either resemble the simple hairs with the exception of the terminal cell, which is glandular, or they consist of a short pedicel bearing a pluricellular gland.

Diagnostic Characters.—

(*a*) The large epidermal cells, with wavy walls and striated cuticle.

(*b*) The stomata, surrounded by three or four cells, one of which is smaller than the others.

(*c*) The cells filled with sandy crystals of calcium oxalate.

(*d*) The absence of pericyclic fibres.

(*e*) The bicollateral bundles (in the section only).

Henbane Leaves

Source.—The leaves of *Hyoscyamus niger,* Linn.

Preparation and Examination.—Prepare and examine henbane leaves as directed for foxglove.

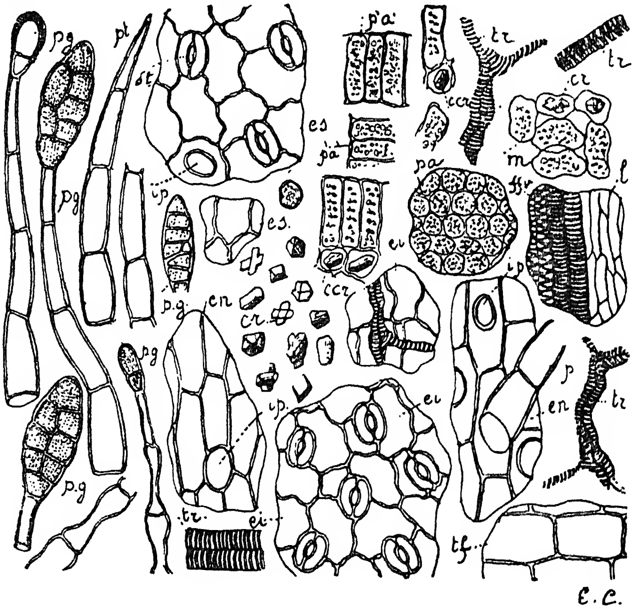


FIG. 80.—Powdered Henbane Leaves. *ccr,* crystal cells; *cr,* crystals of calcium oxalate; *ei,* lower epidermis; *es,* upper epidermis; *ffv,* portion of fibrovascular bundle of midrib; *ip,* scar of fallen hair; *m,* spongy parenchyma; *p,* portion of hair; *pa, p'a',* palisade cells; *pg,* glandular hairs; *pt,* simple hairs; *st,* stomata; *tf,* cortical parenchyma of midrib; *tr,* tracheids and vessels. ×240. (Greenish and Collin.)

The epidermis of both surfaces is composed of cells with very wavy walls and smooth cuticle; they vary from 40 to 100 μ in length. Both surfaces bear simple and glandular hairs, as well as broadly oval stomata. The stomata average about 23 to 27 μ in length. The simple hairs are uniserial and conical, and have thin walls. The glandular hairs are usually long, uniserial, and terminate in a small bicellular gland, or frequently in a large ovoid pluricellular one.

The mesophyll is heterogeneous and asymmetrical; the cells of the spongy parenchyma often contain prismatic crystals of calcium oxalate; in this respect henbane differs conspicuously from stramonium and belladonna, which contain chiefly cluster crystals and sandy crystals respectively.

The midrib is biconvex. The wood is curved, and has bast both above and below it, bicollateral bundles being constant in the natural order Solanaceæ. Neither bast not pericycle contains any lignified elements.

Diagnostic Characters.—

(*a*) The remarkable glandular hairs.
(*b*) The calcium oxalate, mostly in prisms.
(*c*) The epidermal cells with wavy walls.
(*d*) The stomata surrounded by three or four cells, one of which is smaller than the others.
(*e*) The absence of pericyclic fibres.

Identification of an Unknown Powder as that of a Leaf

Having now examined several leaves and their powders, the student must inquire by what means he may identify an unknown powder as that of a leaf.

The following are the most reliable characters of a leaf powder:

(1) The presence of an epidermis with stomata, hairs, &c.
(2) The presence of palisade tissue.
(3) The presence of abundance of chlorophyll or of the brown substance derived from it.
(4) The presence of veins and veinlets.

The identification of the botanical source of an unknown leaf is extremely difficult unless the leaf happens to be one frequently used as a food or a drug. Solereder[1] has given a large number of accumulated facts which may be of service in this respect.

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[1] *Anatomie der Dicotyledonen.*

Finally, it may be observed that, if a powder has been prepared from leaves only, the following tissues or cell contents should be absent:

(1) Cork cells (with few exceptions, such as eucalyptus leaves).
(2) Aleurone grains.
(3) Fat and reserve starch (with few exceptions).
(4) Large proportion of lignified tissue.
(5) Large, thick-walled vessels.

SECTION VIII

FLOWERS

After the student has examined a number of leaves and made himself acquainted with the methods recommended, he may proceed to deal with certain parts of a flower. For the purposes of this work, only the most highly organised flowers of the group of Angiosperms need be considered. These consist of calyx, corolla, stamens, and pistil.

The calyx is usually a leafy organ and closely resembles the leaf in structure. The same methods of treatment and examination that have been recommended in Section VII may also be adopted here. Attention should be particularly directed to the epidermis, to the stomata, and to the presence or absence of hairs, to the various forms of oil cells and glands, internal or external, and also to the calcium oxalate, which is sometimes present in considerable quantity.

The corolla is usually thinner than the calyx and simpler in structure, so that transverse or longitudinal sections may often be dispensed with. The epidermis is generally highly characteristic and requires careful examination. The outer wall is commonly very thin and often striated or papillose. The cells themselves usually contain yellow, red or orange chromoplasts, or coloured cell sap. The mesophyll is much reduced and the fibrovascular bundles are small; nevertheless, characteristic secretory tissue may be found in it.

The most important parts of the stamen are the pollen grains and the endothecium of the anther. The outer membrane of the former may be smooth or, more commonly, bear ridges, projections, spines, &c., of the most varied nature.[1] They are easily identified and may be accepted as evidence of the presence of a flower, although an occasional grain may find its way on to the leaf of another plant; sometimes the genus to which the flower belongs, or even the species, may be determined from the characters of the pollen grain alone. The cells of the endothecium of the anthers bear remarkable spiral, annular, or variously reticulate thickenings which, though present in comparatively small number, may usually be found if a flower is present.

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[1] For illustration of a large number of pollen grains see Hugo Mohl, *Ueber den Bau und die Formen der Pollenkörner*. Bern, 1834.

The structure of the pistil is less important; the papillose stigma is the most characteristic part of it.

Saffron

Source.—The dried stigmata with part of the style of *Crocus sativus,* Linn.

Preparation.—Decolourise a few saffron stigmata by repeated maceration in water, or water to which a little ammonia has been added, and transfer them to 80 per cent. alcohol.

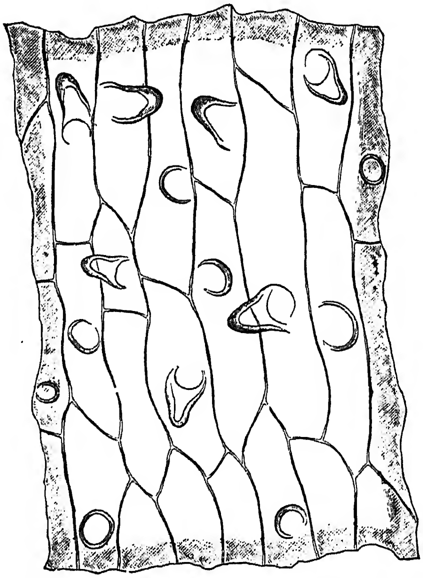

FIG. 81.—Saffron, surface view of epidermis of stigma. (Moeller.)

Examination.—Mount one or a portion of one in water, replace the water by solution of chloral hydrate diluted with an equal volume of water, cover and examine.

The epidermis consists of long, narrow cells with very delicate, often (especially in the lower part) wavy walls; on part of the surface of the stigma these cells bear small papillæ (fig. 83, *u.e.*). At the apex the epidermal cells develop

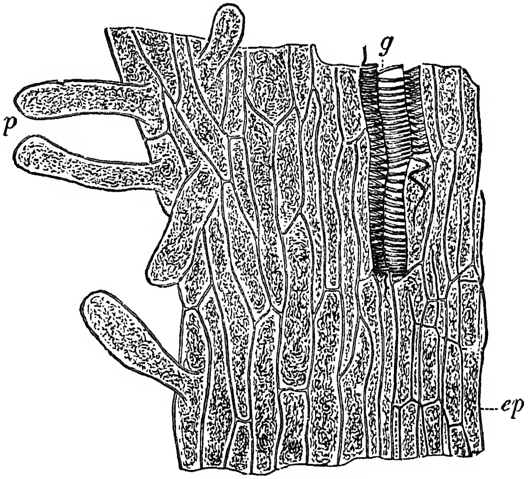

FIG. 82.—Portion of Saffron stigma near the margin. *ep*, epidermis; *p*, papillæ; *g*, spiral vessels. (Moeller.)

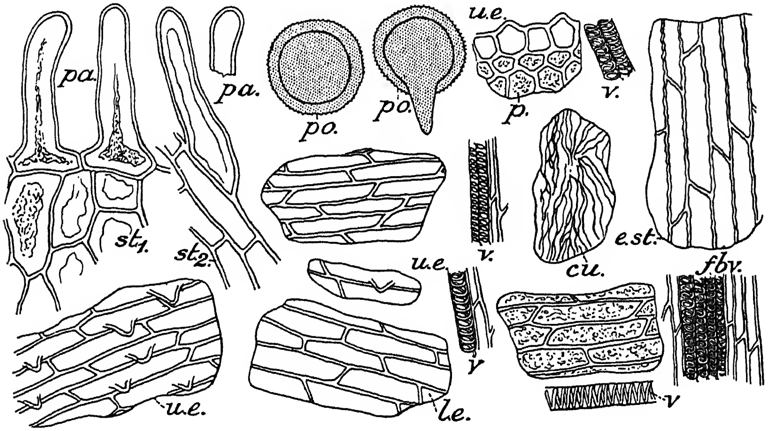

FIG. 83.—Powdered Saffron. *cu.*, cuticle of stigma; *e.st.*, epidermis of style; *fbv.*, fibrovascular bundle; *le*, lower epidermis of stigma; *pa.*, papillæ at apex of stigma; *po.*, pollen grains; *st.*$_1$, upper extremity of stigma; *st.*$_2$, upper margin of same; *u.e.*, upper epidermis of stigma; *v.*, vessel. ×150. (After Greenish and Collin.)

into large projecting papillæ (200 μ long, 20 μ wide), to which large (100 to 200 μ) pollen grains often adhere. Delicate fibrovascular bundles, increasing in number as the stigma broadens, traverse the tissue longitudinally (compare fig. 82).

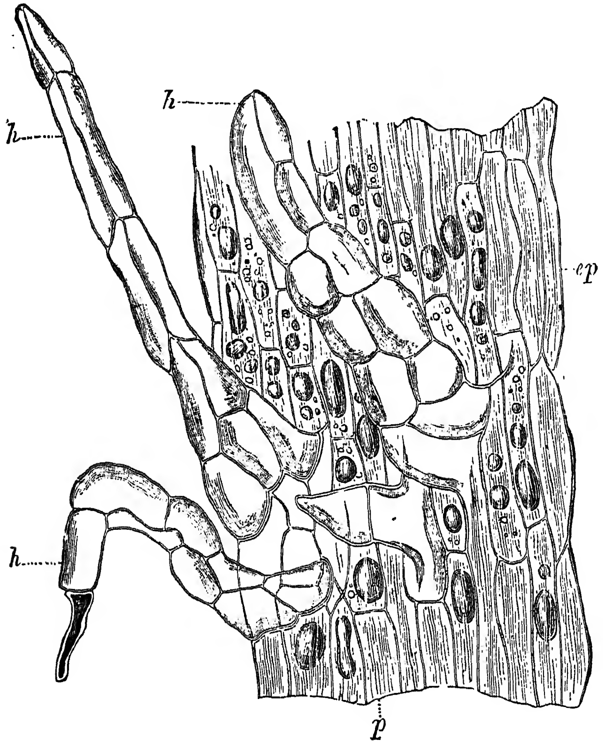

FIG. 84.—Corolla of Calendula. *ep*, epidermis with striated cuticle; *h*, hair; *p*, parenchyma with globules of oil. (Moeller.)

The tissue below the epidermis consists of strongly elongated parenchymatous cells.

Mount a preparation in chlorzinciodine; the cell walls turn blue, the cuticle, which often readily separates, yellow.

Focus the upper surface of one of the pollen grains; it appears granular; scattered on the granular surface are minute warts. Focus downwards until the optical section is reached; the wall appears thick. Here and there a pollen grain with protruded tube may be observed.

Mount a stigma in chloral-iodine; the stigma is free from starch, but very minute grains may be detected in the pollen grains.

Mount a portion of a dry stigma in liquid paraffin; the colouring matter is seen to be in homogeneous, yellow masses; there is no calcium oxalate and but little fixed oil.

Examine another in water; the colouring matter readily dissolves. Irrigate with strong sulphuric acid; it turns deep blue, passing rapidly to violet and brown.

Examination of the Powder.—Examine powdered saffron in the same way.

Strew a little powdered saffron on a drop of sulphuric acid, cover and examine at once. Or cover a little of the powder with a coverslip and irrigate with sulphuric acid. Use the low power. Every particle surrounds itself with a blue liquid, the colour rapidly passing to violet and brown.

Adulterations.—Among the sophistications and substitutions to which saffron is liable, calendula florets (feminell) and safflower must be mentioned and should be examined.

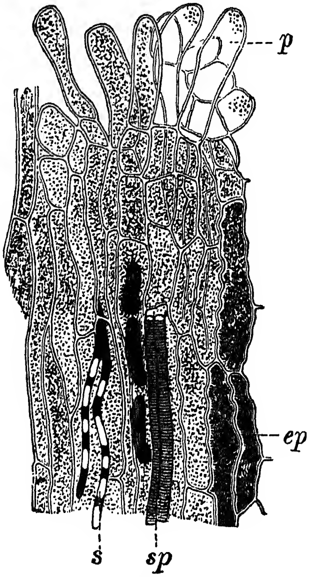

FIG. 85.—Corolla of Safflower. *ep*, epidermis; *p*, papillæ; *s*, secretion-tubes; *sp*, spiral vessels. (Moeller.)

Calendula Florets.—Allow a few calendula florets to expand in water; examine in this medium (or in solution of chloral hydrate). Factitiously coloured calendula (feminell) has a carmine red colour, quite different from that of saffron. The epidermal cells are elongated, the cuticle is delicately striated, and the parenchyma beneath contains numerous large, often yellowish oil globules. Near the tube of the corolla large, characteristic, multicellular hairs occur (fig. 84).

The pollen grains, like most of the pollen grains of composite flowers, are spiny.

Safflower.—Allow a few safflower florets to expand in water. The long, narrow corolla tube divides into five long, narrow segments. Within the tube are the yellow, syngenesious anthers.

Separate with a needle the corolla segments and transfer them to chloral hydrate; the colour changes to yellow and they become transparent. Examine first under the low and then under the high power. The epidermal cells are narrow, elongated, and often (lower portion) have very sinuous walls; these cells develop, at the tip only of the segment, into papillæ; with careful observation very delicate transverse walls may be seen. Narrow fibrovascular bundles similar to those of saffron are also to be seen; they are accompanied by characteristic secretion tubes containing a dark reddish-brown secretion, usually in distinct portions. Many of the transverse walls of the cells near the tube of the corolla exhibit characteristic rod-like thickenings.

The stamens also consist largely of elongated cells passing gradually into isodiametric; many, especially the shorter ones, are reticulated. Characteristic pollen grains, smaller than those of the saffron and with short spines, are usually to be found.

Mount another portion in water; the colouring matter is insoluble.

SECTION IX

BARKS

INTRODUCTION

The structure of the wood, and also that of the young stem in which no very far-reaching secondary changes have been produced, has been considered in Section V and VI. The student should now direct his attention to the bark. Before doing so, it is very desirable that he should study that section of his text-book of botany which deals with the formation of this part of the stem.

It will be convenient here to use the term ‘ bark ’ in its widest signification, and to understand by it all those tissues of the stem that are exterior to the cambium, no matter whether they are primary, secondary, or tertiary—that is, whether they have been produced by the division of the cells of the growing point, or whether they have been produced from a cambium (secondary tissues), or from a secondary cambium (tertiary tissues).

The changes by which the bark is developed from the tissues of the young stem are brought about largely by the activity of two circles of merismatic cells, an inner circle or cambium and an outer circle or phellogen (cork cambium). The cambium produces wood towards the interior of the stem and bast towards the exterior ; the phellogen produces cork towards the exterior and usually phelloderm towards the interior, but the latter tissue is often developed sparingly or not at all.

The former of these two circles invariably develops between the primary bast and primary wood, but the point at which phellogen forms is subject to considerable variation. Sometimes it is the epidermis, frequently the subepidermal layer of cells, but it may also form in any layer of the cortex, in the endodermis, or in the pericycle. As the formation of cork cells by the phellogen causes the tissue exterior to it to perish and often disappear, it is evident that the structure of the bark may thereby be considerably modified.

Much change may also be induced by the formation of secondary phellogens. These may arise in the phelloderm, in the cortex, or in the secondary bast, and, as with the primary phellogen, their production results in the death and ultimate destruction of the tissues external to them. The mass of external tissue thus formed includes all the layers of cork, together with varying quantities of cortical tissue, pericycle, and bast. It has been called by botanists 'bark,' but as the term is in common use to designate all the tissues of the stem exterior to the wood, this mass of external protective tissue, the *outer* portion of the bark, may be specified as 'outer bark.'

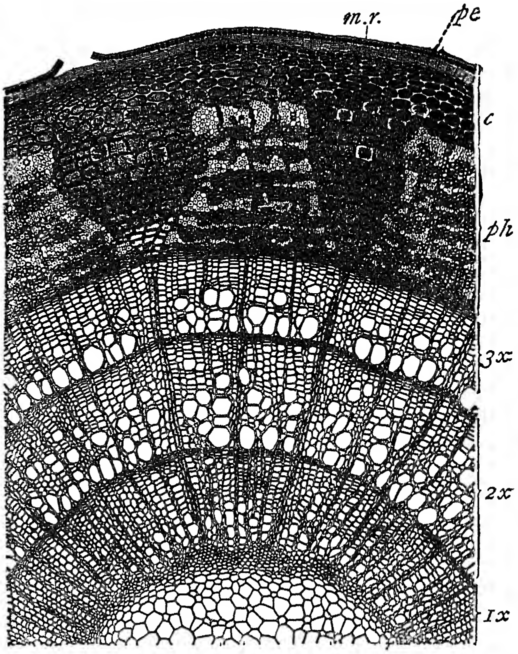

Fig. 86.—Transverse section of a twig of lime, three years old. *1x*, *2x*, *3x*, the successive annual rings of wood; *ph*, phloem (bast); *c*, cortex; *m.r.*, primary medullary ray; *pe*, periderm (cork). (After Kny.)

In the examination of a bark the following tissues may therefore be met with :

(1) Cork.
(2) Phelloderm.
(3) Outer bark.
(4) Cortex.
(5) Bast (or bast ring).

In order to be able to describe a section of a bark correctly, it is necessary to know how these tissues may be recognised and delimited.

Were an easily recognisable endodermis present, as is the case with many herbaceous stems, it would be very desirable to identify it, and thus at once determine the inner limit of the cortex and the outer limit of the bast ring. Unfortunately, the endodermis is seldom to be found ; in many barks it has been thrown off by the formation of secondary phellogens within it ; in others, the growth of the bark has rendered it indistinguishable. Recourse must, therefore, be had to other means of determining the inner limit of the cortex, and these, while often available, do not allow of the same precision as the identification of the endodermis would.

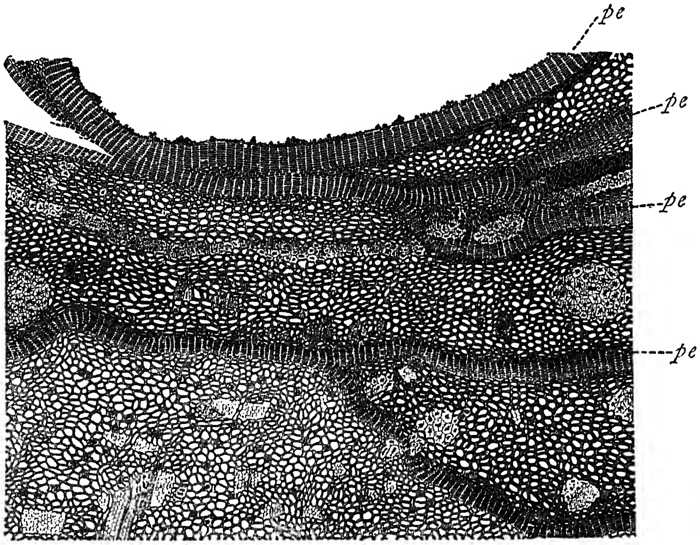

FIG. 87.—Outer Bark of Oak, transverse section. *pe*, periderm (cork) layers arising at different depths in the cortex. (After Kny.)

In the first place it must be observed that sclerenchymatous fibres frequently develop in the pericycle. These fibres often differ in shape, &c., from the fibres of the secondary bast. They are often designated 'primary bast fibres,' although they have not had their origin in the bast, but in the pericycle. Since the endodermis is immediately exterior to the pericycle, these pericyclic fibres mark, more or less accurately, the position of that tissue. Moreover, the cells of the pericycle often lignify and form a ring of sclerenchymatous cells, in which the pericyclic fibres are commonly included. Such a ring of sclerenchyma is found in oak bark, witchhazel bark, &c., and indicates the position of the endodermis in these drugs.

In many barks, however, no such ring of sclerenchyma or bundles of pericyclic fibres are to be found. In these cases it is advisable to trace the course of the medullary rays from the cambium towards the cork; the tissue through which they pass is undoubtedly part of the bast ring, but a region, usually narrow, is always to be found which cannot be definitely assigned to either cortex or bast.

The student is advised to make a preliminary examination of the transverse section under a low power, and to make a diagrammatic sketch of it, upon which the limitations of the tissues present should be indicated. He can then proceed to examine and describe each tissue in succession (compare the diagrammatic sketch of Cascara bark, fig. 88).

Cork.—The cells of which this tissue is composed are developed from a single layer of cells (phellogen) by repeated tangential division. They are, consequently, arranged in regular radial rows, an arrangement which is usually easily visible, even in cork of considerable age. They are often tangentially elongated, and have thin, strongly refractive walls. They often contain an amorphous brown or red substance that reacts for tannin, and their walls are also frequently yellowish brown in colour. The walls of cork cells are always suberised and very frequently lignified. In surface view they are polygonal and nearly isodiametric.

While these are the characters of typical cork cells, variations from the type often occur. In some barks the cell walls exhibit an appreciable thickening, which may be uniform or restricted to the outer or to the inner wall. In the cork of cascarilla bark crystals of calcium oxalate are embedded in the inner wall, a most unusual occurrence, and one, therefore, that materially aids in the identification of the drug.

Phelloderm (or Secondary Cortex).—This tissue is also developed from the phellogen, but on its inner surface. The cells of which it is composed are arranged, as cork cells are, in radial rows. They are, however, easily distinguished from cork cells by their walls not being suberised and by the absence of colouring matter, while the arrangement in radial rows distinguishes them, at least when young, from the cells of the cortex upon which they abut. When, however, the development of phelloderm is large, the radial arrangement of the cells becomes obliterated, and this tissue cannot then be distinguished from the cortex.

Cortex.—The cortical tissue, or primary cortex, so called to distinguish it from the phelloderm or secondary cortex, is composed of parenchymatous tissue, the cells of which are usually tangentially elongated and exhibit more or less well-marked intercellular spaces. Near the cork it often differentiates into strands of collenchymatous tissue. It often contains sclerenchymatous cells, either isolated or in groups; sclerenchymatous fibres are less frequently to be found. Secretory tissue of various kinds—such as oil cells, oil glands, &c.—may be present, and contribute to the identification of the drug. Among cell contents, chlorophyll is often contained in the outer layers, while starch grains and calcium oxalate crystals are frequently to be found.

Bast Ring (including the Pericycle).—Abutting upon the cortex there is to be found, in many barks, a more or less continuous ring of sclerenchymatous cells, with which groups of fibres are often associated.

Next to the sclerenchymatous ring comes the bast ring or bast zone. This tissue is traversed by medullary rays continuous with those of the wood, and may therefore be conveniently divided into medullary rays and bast rays.

The medullary rays of the bast resemble in appearance and contents (starch, calcium oxalate, &c.) those of the wood, but their cell walls remain thin and do not lignify. Towards their outer limit the rays usually widen, and here sclerenchymatous cells, or various forms of secretory tissue, such as oil cells, oil glands, &c., may often be found.

The essential components of the bast rays are sieve tubes and bast parenchyma, but with these there may be also associated secretory tissue and sclerenchymatous cells or fibres.

Sieve tubes may be distinguished from bast parenchyma by the difference in the size of the transverse section, and by the difference in the thickness of the wall. Usually the sieve tubes are larger, and have thicker and less regular walls, which also often exhibit a bluish colour. Sometimes the companion cell that is attached to the sieve tube can be detected, and, when the tubes are of large size, portions of the sieve plate can often be observed. Should these, as is commonly the case, be covered with callus, the latter can be stained with appropriate reagents. Digestion in the water-bath with solution of caustic potash, or with a mixture of equal volumes of hydrochloric and nitric acids, so loosens the cells that the sieve tubes can by this means be isolated and examined, but it is only in comparatively few instances that they possess any decided diagnostic value, though they frequently afford important indications of the nature of a powder.

To sclerenchymatous cells and fibres, on the other hand, if present, considerable importance is to be attached. The forms of the cells, of their sections, the thickness of the walls, the extent to which lignification has taken place, should be ascertained, and the distribution of the cells should be studied. Sclerenchymatous cells are more commonly grouped, but the fibres may occur either isolated or in groups ; in the latter case the arrangement of the groups must be noted.

It is very desirable that the student should be quite familiar with the characters that distinguish sclerenchymatous fibres from sclerenchymatous cells, both in transverse and radial sections, and also when isolated. The following characters should be carefully noted:

In transverse section sclerenchymatous fibres are usually rounded or polygonal, and much thickened, so that the cavity is often reduced to a point; they are comparatively rarely oval and seldom bear pits. Sclerenchymatous cells, on the other hand, are often irregularly rectangular in shape, have thick, striated walls, which are traversed by branching pits ; the cavities are sometimes large, but, if much reduced, they are usually linear, not punctiform.

In radial section, and also when isolated, typical fibres are distinguished by their great length and tapering ends ; the pits are scattered, and have the form of narrow slits, usually arranged in a left ascending spiral. Sclerenchymatous cells have much the same shape in radial as in transverse sections; they have square or rounded ends, and circular pits.

Although these characters sufficiently distinguish typical sclerenchymatous cells from typical fibres, intermediate forms are not unfrequently found, as, for instance, in cinchona bark. Such intermediate forms may be referred to the class of fibres, if their pits are narrow and oblique, to the cells, if they are rounded, but in each case the elements in question should be accurately described.

It sometimes happens that, in the older (outer) portions of the bast ring, the sieve tubes that have ceased to be active are pressed by the growth of the bark into strands in which little or no structure can be observed. As the sieve tubes often assume a tangential arrangement, these strands of collapsed sieve tubes are often prolonged to a considerable extent tangentially, and may be very conspicuous in the transverse section of the bark (canella, cinnamon, &c.).

Outer Bark.—When this tissue is present it is usually dark brown in colour, and consists of dead portions of the cortex or bast ring, alternating with layers of cork. The elements of these respective tissues may readily be identified, although somewhat modified by the changes they have undergone.

Diagnostic Characters.—The following are the chief features to which the student should particularly direct his attention, as being of considerable diagnostic value.

1. *Cork.*—The extent to which this tissue is produced varies in different barks. Important details are to be found in the size of the cells and the nature of the walls; whether the latter are thin or thick, and whether the thickening, if present, is uniform on all the walls. The nature and colour of the contents should also be noted.

2. *Phelloderm.*—In so far as this tissue is distinguishable from the primary cortex, it seldom possesses any distinctive features. In canella bark the cells of the phelloderm are characterised by their very pronounced one-sided thickening.

3. *Cortex.*—The parenchymatous cells of the cortex do not, as a rule, present any remarkable features, but their contents may (starch, calcium oxalate, &c.). More important is the presence of secretory tissue of any kind (oil cells, oil glands, &c.) or sclerenchymatous cells or fibres. Any of these should be subjected to careful scrutiny.

4. *Bast Ring.*—This tissue usually constitutes the major part of commercial barks, and in it distinguishing characters are usually found.

The presence or absence of sclerenchymatous cells or fibres should first be noted. If either be present, the shape of the cells, the thickness and nature of the walls, the striations, pits, &c., should be studied. The amount and distribution of these elements should also be examined; whether they are isolated or in groups, and, in the latter case, whether the groups are radially or tangentially arranged. While the arrangement is a valuable character for the entire drug, it is less valuable for the powdered drug; in the latter case, the details of the individual elements and their occurrence as isolated cells or as groups should be determined.

The presence or absence in the bast ring of any form of secretory tissue is also most important. The shape of starch grains that may be present, the form assumed by the calcium oxalate, all contribute their quota to the identification of the drug. The characters of the sieve tubes, their distribution, size, the position of the sieve plates, &c., should also be determined.

Powdered Barks.—Although the various official barks exhibit in section notable differences, these differences frequently depend upon the arrangement of the tissues and elements present, rather than upon any distinctive characters of the cells themselves. These differences in arrangement are naturally lost in the powdered drugs, and it becomes necessary to devote minute study to the details of the cells that are present.

Perhaps the most important of all these are the bast fibres, few barks (e.g. canella and pomegranate) being devoid of them. These resist the action of the grinding machinery, and, though they may be and often are broken, especially in very fine powders, they usually exhibit their longitudinal aspect, and are easy to detect and identify. Their occurrence, singly or in groups, their size and shape, the thickness, colour, and striation (if any) of their walls, and the character of the pits should be studied. The number in which they are present should also be observed. They closely resemble the sclerenchymatous fibres of the wood, but are usually more strongly thickened.

Next in importance to the bast fibres are the sclerenchymatous cells, which, however, are more frequently absent than bast fibres. They commonly resist the action of the drug mill, and are found in the powder in a more or less intact state, either isolated or two or three together. Sometimes here also the character of the secondary thickenings (canella, cassia, cinnamon) and other details may be useful.

The presence of sieve tubes is important as an indication of the nature of the powder. Especially in the coarser particles they may be found without great difficulty, particularly if precaution is taken to bleach the powder and stain the callus plates. The size of the sieve tubes and the arrangement of the sieve plates, whether transverse or oblique, simple or complex, should be noted.

Most bark powders contain cork tissue, those alone from which it has been stripped being more or less completely destitute of it. Cork offers considerable resistance to the drug mill, and is usually found in small flattened portions that expose their surface view to the observer. The size of the cells, thickness of the walls, nature and colour of contents, may all afford useful confirmatory evidence.

The parenchymatous tissue of the barks is not, as a rule, characteristic, except as regards the cell contents. These are mostly starch and calcium oxalate, and their presence, as well as the forms they affect, should not be neglected.

The starch grains are usually small and by no means so characteristic as reserve starch. Colouring matter is often present and may be characteristic.

Fragments of cortical parenchyma may often be recognised by the comparatively large size of the cells and the presence of intercellular spaces; the cells of the bast parenchyma are usually narrower and axially elongated and exhibit no intercellular spaces. In fine powders both tissues are much disintegrated, many fragments of the cell walls being present.

Secretory tissue has here, as with the leaves, distinct diagnostic value. Large oil glands, and often oil cells, are so destroyed by the grinding as to be difficult of detection, though the suberised wall of oil cells may be recognised by suitable staining (Soudan red). Elongated secretion cells, especially if filled with a secretion of characteristic colour, are, on the other hand, more easy to find.

The colour of the particles of a powder may also offer a means of identifying it which should be utilised.

General Scheme of Examination.—The following general scheme may be adopted for examining barks :

1. Preparation of transverse sections ; examination of the tissues present, and determination of their distribution ; preparation of a diagrammatic sketch indicating these, and sketches of portions of the section on a scale large enough to include all details.
2. Preparations and examination of radial sections ; sketches of important details.
3. Isolation of the tissues by maceration, either (*a*) with caustic potash, (*b*) with potassium chlorate and nitric acid, or (*c*) with hydrochloric and nitric acids.
4. Examination of the powder, both coarse and fine.

Cascara Sagrada

Source.—The bark of *Rhamnus Purshianus*, D.C.

Preparation and Examination of Transverse Sections.—Select a thin piece of bark, and cut from it several small pieces about 1 cm. long and 3 to 5 mm. wide. Soften them by exposing them to a moist atmosphere for twelve or twenty-four hours. Embed in pith, as already described, and cut transverse sections. Place these at once in alcohol to free them from colouring matter and air.

Transfer one or two of the thinnest to a drop of water on a slide, spread out with the needles, add a drop of glycerin, and examine. If the structure is not sufficiently distinct, mount another section in chloral hydrate.

On the outside a comparatively narrow layer of small cork cells can be seen, and recognised by their reddish-brown contents. Here and there the cork may be covered by a whitish coating of lichen, the structure of which may be passed over. Trace the medullary rays from the cambium towards the cork as far as possible ; there is no sharp line of demarcation between the secondary bast and the cortex. Prepare a diagrammatic sketch of the section, in which the extent and relative positions of these tissues (but not the constituent cells) are shown (compare fig. 88). Stain one or two sections with phloroglucin and hydrochloric acid. Observe groups of cells which are stained deep red. Some of these are groups of sclerenchymatous cells, others are groups of sclerenchymatous fibres (bast fibres); in the latter the individual cells are easily distinguished; they are small, rounded or polygonal, and almost completely filled with secondary thickening. Notice that the groups of bast fibres are narrow, tangentially elongated, and stretch from one medullary ray nearly, or quite, to the next; they are arranged in fairly regular tangential lines. Introduce the groups of sclerenchymatous cells and fibres into the sketch, outlining them only, but keeping the sizes and positions of the groups correct. Key the sketch with letters indicating the nature of each of the tissues.

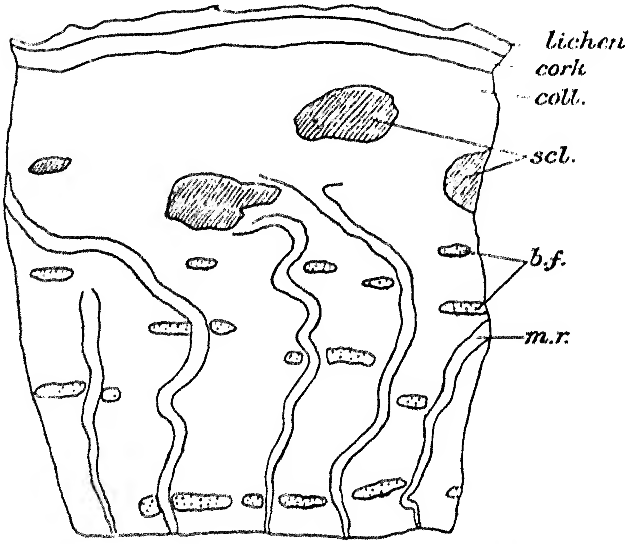

FIG. 88. Cascara Bark, diagrammatic transverse section. *b.f.*, bast fibres; *coll.*, collenchymatous tissue; *m.r.*, medullary ray; *scl.*, sclerenchymatous cells.

Next proceed to examine each of these tissues more in detail.

1. *Cork.*—This consists of several rows of narrow, flattened cells with thin walls and dark reddish-brown contents. The arrangement of the cells in regular radial rows indicates their formation from a phellogen. The walls of the cork cells are suberised (reactions with sulphuric acid and with Soudan red). The innermost row of cells is the phellogen (provided no phelloderm has formed).

2. *Phelloderm.*—This tissue is developed, at most, in small quantity only; the cells are distinguished from the phellogen cells by having thicker walls, from the cork cells by not being suberised, and from the cells of the cortex (usually) by their arrangement in radial rows.

3. *Cortex.*—The cells are larger than those of the phelloderm, and are not arranged in radial rows. Near the cork they may exhibit collenchymatous thickening of the wall and be tangentially elongated, but towards the bast ring they are usually rounded and thin-walled, with intercellular spaces. The pits are distinct; some of the cells have walls that are more or less evidently reticulately thickened.

While the outer limit of this tissue is easy to determine in the bark under examination, the inner limit is not. The endodermis, which in young stems sharply delimits the cortex, has long since disappeared, and the cortex passes insensibly into the bast ring. Since the former contains no medullary rays, it is quite certain that the tissue in which these can be traced is part of the bast ring. But the medullary rays commonly become indistinct near the outer limit of the bast ring, and this indication is therefore not a precise one. Here and there (but by no means in all sections), not far from the cork, a group of flattened oval fibres with moderately thick walls may be found. These are pericyclic fibres, and indicate fairly accurately the limit of the cortex.

The cortex contains also groups of sclerenchymatous cells. These groups are usually irregularly rounded or tangentially elongated; the cells of which they consist vary very much in size, but agree in having very thick and conspicuously striated walls, through which branching pits run; the lumen is often nearly filled up, and the cells, when stained with phloroglucin and hydrochloric acid, assume a deep pink colour.

The parenchymatous cells of the cortex contain chlorophyll and also calcium oxalate; the latter is usually in rosette crystals except near the sclerenchymatous cells, where prisms are commonly present. There is little or no starch to be found.

4. *Bast Ring.*—As already indicated, the tissue referred to under this name may contain the pericycle and the primary and secondary bast. Its external limit is very ill-defined. In the bark under examination, if the groups of flattened, oval, pericyclic fibres cannot be identified, there remains no course

open but to trace the medullary rays as far as possible and to speak of the whole of this tissue as the bast ring. It usually consists almost entirely of secondary bast. The latter may be divided into medullary rays and bast rays. In cascara bark the former exhibit the usual characters; they are commonly from two to four cells wide, and contain a yellow substance, which, however, is easily soluble in alcohol, and therefore absent from sections treated as indicated on p. 158.

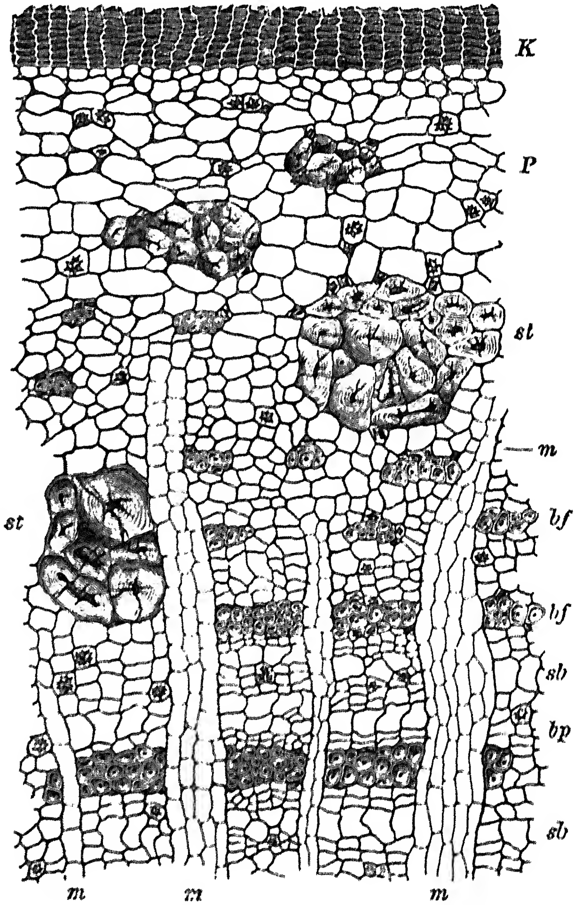

FIG. 89.—Cascara Bark, transverse section. *bf*, bast fibres; *bp*, bast parenchyma; *K*, cork; *m*, medullary rays; *P*, cortex; *sb*, sieve tubes; *st*, groups of sclerenchymatous cells. (Moeller.)

The bast rays are important, and require very careful examination. Note in them occasional groups of sclerenchymatous cells such as have already been described. Note also the presence of narrow tangentially elongated groups of bast fibres. The individual fibres are polygonal or nearly rounded in outline (in transverse section), uniform in size, and so much thickened that the cavity is reduced to a point. In the phloroglucin preparation they are stained red, but not so strongly as the sclerenchymatous cells; they are, therefore, with the exception of the middle lamella, less strongly lignified. These fibres exhibit occasional pits which, however, are unbranched. The student should carefully compare groups of fibres with groups of sclerenchymatous cells. The two cell forms can usually be distinguished by the transverse section alone, but more definite information is afforded by a longitudinal section.

Notice, in the parenchymatous cells that abut on the fibres, small prisms of calcium oxalate.

The other tissues present in the bast rays of cascara bark are sieve tubes and bast parenchyma. Of these, the former are the more important. They are a never-failing constituent of barks, and must therefore be carefully examined.

Stain a section that includes the rows of cells near the cambium with corallin soda (preparing a fresh solution by dissolving a trace of corallin in a little 25 per cent. solution of sodium carbonate), and examine it in the reagent. Note, in some of the cells near the cambium, masses that assume a brilliant pink colour. These are the masses of callus deposited on the sieve plates, and the cells in which they occur are sieve tubes. The latter are larger than the parenchymatous cells of the bast, and usually alternate with them in tangential bands (fig. 89, *sb*). Examine an unstained section; the sieve tubes can now be generally identified and the sieve plate often distinguished. Sometimes this plate is transverse, but more often oblique; hence a portion only of the sieve plate is usually seen. On many sieve plates no callus has been formed, and these do not stain with corallin; they may, however, be identified by their larger size and rather thicker walls, which are less uniform than those of the bast parenchyma.

Examine a transverse section mounted direct in water (without, therefore, previous treatment with alcohol). Note the granular, dingy yellow contents of the parenchymatous cells of the cortex, bast, and medullary rays. Irrigate with solution of caustic potash; the yellow substance instantly dissolves, with production of a bright purple coloration.

Having thus thoroughly examined the section, the student should sketch small portions on a comparatively large scale; they should include:

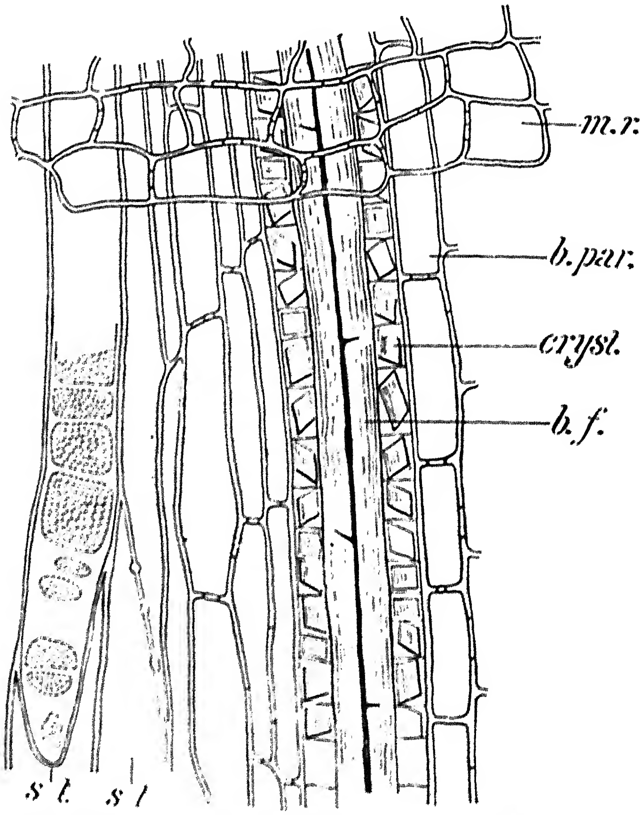

FIG. 90.—Cascara Bark, radial section. *b.f.*, bast fibre; *b.par.*, bast parenchyma; *cryst.*, crystal (of calcium oxalate); *m.r.*, medullary ray; *s.t.*, sieve tubes. × 450.

1. A portion of the cork, taking care to reproduce accurately the shape of the cells and thickness of the walls;
2. A portion of the bast ring, including a group of bast fibres, the sieve tubes and bast parenchyma, and the medullary ray on either side;
3. A group of sclerenchymatous cells.

Radial Sections.—Next cut radial sections and treat in the same way. Identify and examine the elements that have been seen in the transverse sections.

The cork cells present an appearance very similar to the transverse section, but the collenchymatous cells of the cortex have generally a rounded and smaller lumen ; the latter cells are therefore tangentially elongated. The inner rows of cortical parenchyma have thinner walls and rounded section, and show intercellular spaces.

The groups of sclerenchymatous cells also closely resemble those seen in transverse section. The bast fibres, however, are seen to be long prosenchymatous elements, with a very small lumen and few pits, the latter with difficulty visible in chloral hydrate. Each group is bordered by regular rows of small cells, each of which contains a prism of calcium oxalate.

The medullary rays are easily seen in radial sections as plates of radially elongated cells (compare Quassia Wood).

Next identify the sieve tubes, aiding identification, if necessary, with corallin soda. They should be sought for in the cells near to the cambium. They are long wide tubes, showing at intervals oblique sieve plates. They are easily distinguished from the parenchyma of the bast, the cells of which are smaller and shorter, and have thinner walls and square ends, but often have large pores that may be mistaken for small sieve plates. Note also the beaded appearance of the sieve plates in transverse section, and compare with the beaded appearance sometimes exhibited by the bast parenchyma in similar sections.

Isolation of the Elements by Maceration with Potash

Cut from a small piece of bark several longitudinal strips about 1–2 mm. wide. Cut these again transversely into pieces about 3–4 mm. long. Macerate several of these in a water-bath for five to fifteen minutes in a solution of potash containing 2 per cent. of caustic potash. Wash with *distilled* water (to avoid deposition of crystals of calcium carbonate, which might occur if tap-water were used). Transfer one to a slide, tease out the different tissues with the needles, and examine. Nearly all the parenchymatous cells contain a bright purplish colouring matter. The cork is easily identified; it consists of plates (surface view) of polygonal cells with brown contents. The bast fibres are very conspicuous by reason of their yellow colour (produced by the action of the alkali) and accompanying crystals of calcium oxalate. The sclerenchymatous cells are seen as rounded groups of yellow, thick-walled cells. The sieve tubes can also be identified (corallin soda); they often remain connected with one another, if the action of the alkali has not been too energetic.

Sketch a sieve tube, with its sieve plate.

Examination of the Powder.—Reduce a little cascara bark to a coarse powder. Sift this first through a No. 20 and then through a No. 60 sieve, so as to separate it into coarse, medium, and fine powder. Examine the coarse fragments with the lens, and endeavour to pick out some with the needle or brush. Fix them on pith as directed for bearberry leaves, and cut from them sections, which can then be compared with the sections made from the drug itself. The medium powder may be examined in the same way, and part of it may be reduced to a fine powder and treated as described below for that which passes through a No. 60 sieve.

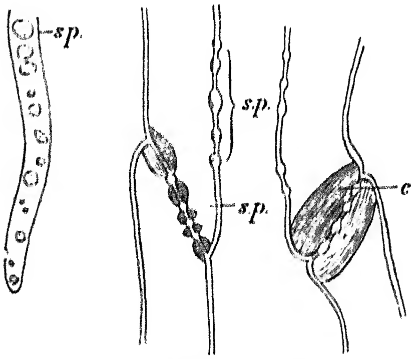

FIG. 91. Sieve Tubes of Cascara Bark. s.p., sieve plates; c, callus. × 320.

Mount a little of the fine powder in water or dilute glycerin, and examine. Note the reddish-brown colour of the contents of the cork cells, and the yellowish colour of those of the parenchymatous cells; add a little solution of potash, and observe in the latter the change to purple.

To another portion of the powder, previously moistened with alcohol and allowed to become nearly dry, add chloral hydrate. This reagent rapidly clears the tissues; with a little care most of the colouring matter can be washed out by irrigation. Observe in this preparation particularly the following details:

1. *Cork.*—The cells are polygonal, and have rather thin walls without intercellular spaces; they have reddish-brown contents.
2. *Sclerenchymatous Cells.*—These are in oval or rounded

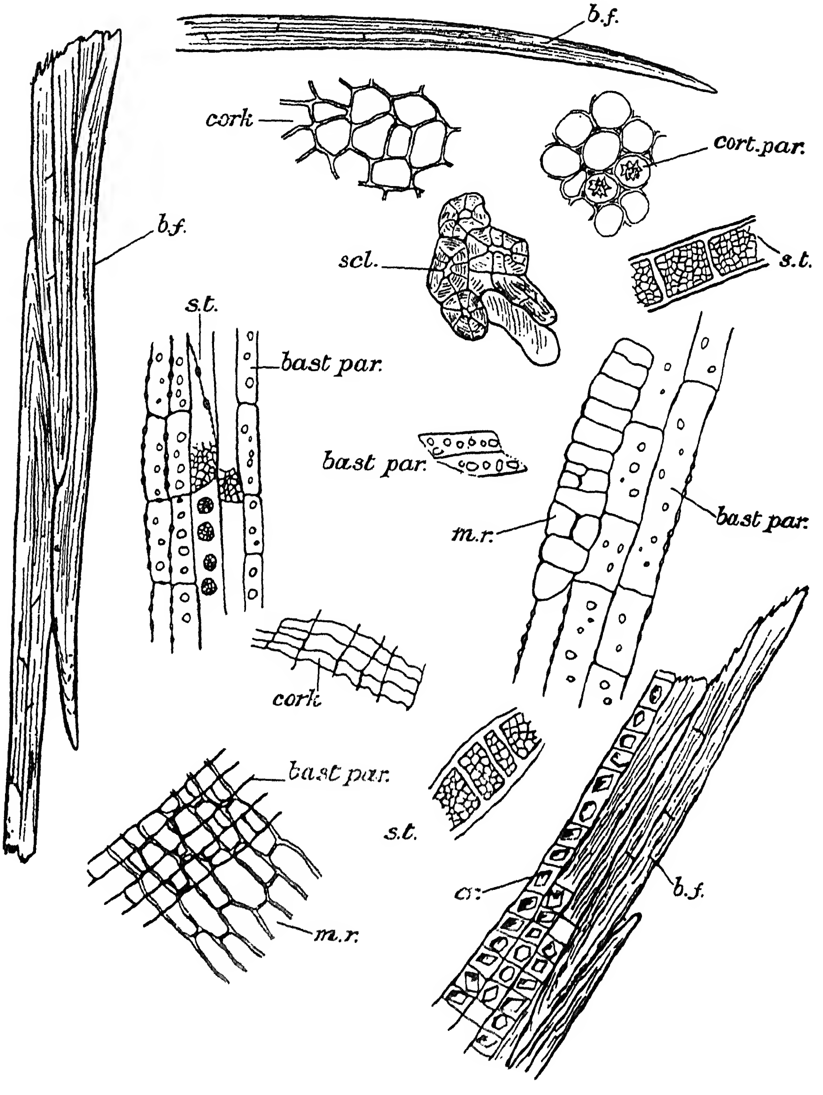

Fig. 92.—Powdered Cascara Bark. *b.f.*, fragments of bast fibres, with and without crystal cells adhering; *bast par.*, bast parenchyma; *cork*, cork, in surface view and section; *cort. par.*, cortical parenchyma, containing rosettes of calcium oxalate; *cr.*, crystals of calcium oxalate; *m.r.*, medullary ray, in tangential and radial section; *scl.*, sclerenchymatous cells in group; *s.t.*, fragments of sieve tubes. × 220.

groups; the walls are very thick, striated, and traversed by branching pits; the details of the individual cells are often difficult to distinguish.

3. *Sclerenchymatous* (*Bast*) *Fibres.*—They are mostly in groups, usually conspicuous by their yellow colour and by the rows of crystals that accompany them; the shape of the fibres can generally be ascertained.
4. *Bast Parenchyma.*—Axially elongated parenchymatous cells, with large rounded pits; the walls in section often appear beaded.
5. *Sieve Tubes.*—These are easily identified by their size as well as by the sieve plates that they contain. They are often accompanied by bast parenchyma, which remains attached to them; they are particularly easy to find in a preparation stained with corallin soda (see below).
6. *Medullary Rays.*—The cells occur in plates and cross the bast parenchyma, with which they often remain associated, at right angles; the transverse walls are pitted.
7. *Cortical Parenchyma.*—Large rounded cells, often with intercellular spaces, and containing here and there cluster crystals of calcium oxalate.

With a little experience these various cells and tissues can be identified without further experiments; but the following preparations, made with a little of the powdered bark that has been freed from colouring matter (either by exhaustion with chloral hydrate or by maceration for a few minutes with solution of chlorinated soda and washing), may be useful to the student.

1. Mount a little of the bleached powder in corallin soda; the callus plates stain pink; by this means the sieve tubes can be identified.
2. Stain a little with phloroglucin and hydrochloric acid; the bast fibres and sclerenchymatous cells stain red.
3. Warm a little with Soudan red, cool, and, after a few minutes, examine; the cork cells stain reddish.

Alderbuckthorn Bark

Source.—The bark of *Rhamnus Frangula,* Linn.

Examination.—Treat this bark exactly as the preceding. Note the following differences:

1. Most of the cork cells contain a bright purplish colouring matter.
2. There are no sclerenchymatous cells in the cortex or bast ring.
3. The colour of the contents of the parenchymatous cells is a rather bright yellow.

In the powder the same differences may easily be observed, and the powders of the two barks may readily be distinguished. In mixtures of the two powders, cascara bark can be identified by its sclerenchymatous cells, and alderbuckthorn by the purple colour of the contents of the cork cells.

Witchhazel Bark

Source.—The bark of *Hamamelis virginiana,* Linn.

Preparation and Examination of Sections.—Select some pieces of the bark, if possible with cork attached; cut small pieces, and soften in a damp atmosphere. Cut transverse sections, and treat them as directed for cascara bark.

The outside layer is cork; the cells of which it is composed are rather large, and have thin walls; they are nearly isodiametric, or, at least, not conspicuously flattened; most of them are empty, but some contain a red-brown amorphous substance. If the cork is well developed, a layer of thin-walled cells, several rows wide, may be found alternating with one or two rows of thick-walled pitted cells.

Within the cork there is usually a phelloderm consisting of several rows of cells. These may be identified as phelloderm by the regular radial rows in which the cells are arranged; this shows that they have originated from a line of merismatic cells.

Mount a section in water, remove the water with filter paper and add solution of chlorzinciodine; the phelloderm cells colour bluish-violet (cellulose reaction), while the cork cells colour yellow (suberin reaction).

The phelloderm passes into primary cortex; the commencement of the latter tissue may in this case (but not always) be recognised by the position of the radial walls, which are no longer continuous with those of the phelloderm, but alternate with them. The inner rows of cells belonging to the primary cortex are often tangentially elongated.

This tissue is distinguished by containing large, well-formed, prismatic crystals of calcium oxalate (the previous barks containing cluster crystals).

Passing through the primary cortex towards the cambium

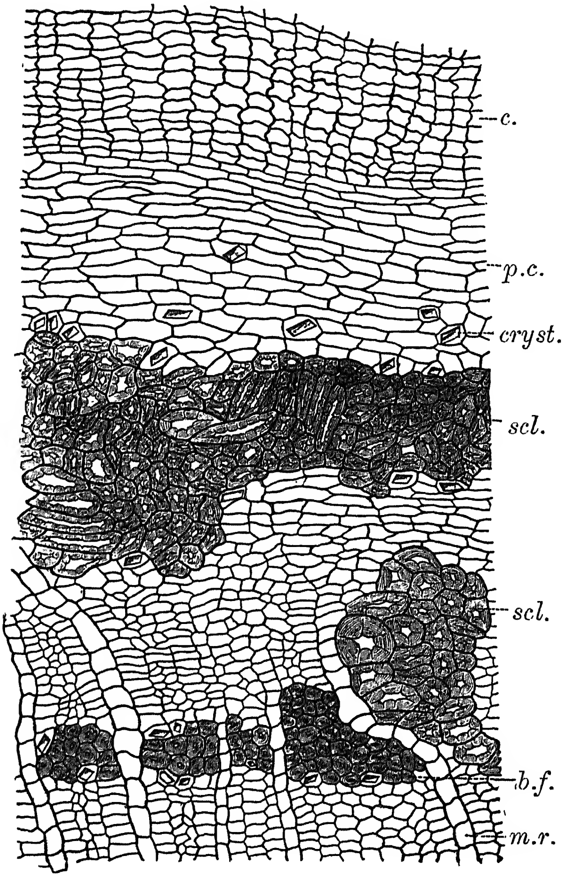

FIG. 93.—Witchhazel Bark, transverse section. *b.f.*, bast fibres; *c.*, cork; *cryst.*, prismatic crystals of calcium oxalate; *m.r.*, medullary ray; *p.c.*, primary cortex; *scl.*, sclerenchymatous cells × 130.

a complete or, sometimes, interrupted ring of sclerenchymatous cells is reached, some of which are very large (100 μ long or even more), many of medium size (40 to 60 μ), some very small (10 to 20 μ). These have been developed from the cells of the pericycle, and they do not belong, therefore, to the primary cortex.

The tissue within the sclerenchymatous ring consists of primary and secondary bast. In this tissue there occur groups of sclerenchymatous cells of varying dimensions; they resemble those of the sclerenchymatous ring.

The medullary rays are one cell wide.

The bast fibres are conspicuous by reason of their number; they are strongly thickened, arranged in tangentially elongated groups, which stretch from one medullary ray to another, and are accompanied by crystal cells.

The sieve tubes and bast parenchyma which compose the remainder of the bast ray resemble those of cascara, but the sieve tubes are smaller, less easy to distinguish, and do not always stain with corallin; they are therefore rather more difficult to identify.

Having thus investigated the structure of the bark as exhibited by transverse sections, mount a section direct in water; note the general absence of colour; some only of the cork cells, of the sclerenchymatous cells, and of the parenchymatous cells contain an amorphous reddish-brown substance. Many appear of a dull, pale, greyish-brown colour.

Irrigate with solution of potash; there is no striking change of colour.

Cut several transverse sections from bark that has been softened by exposing it to a moist atmosphere. Transfer one direct to a drop of dilute solution of ferric chloride; the whole of the parenchymatous tissue, including the medullary rays, especially the parenchyma of the secondary bast, is coloured deep bluish black (reaction for tannin); the cells of the cortex and of the cork are less strongly coloured; the sclerenchymatous cells and bast fibres remain quite colourless.

This reaction indicates that in the drug the tannin is contained in the parenchymatous cells; but, as this substance easily passes from the cells in which it was originally contained into the surrounding tissue, it does not necessarily follow that it was originally present in all these cells.

A number of other substances give a dark bluish or greenish coloration with ferric salts,[1] and the reaction does not, therefore, necessarily indicate the presence of tannin.

Mount another section direct in Braemer's reagent; the parenchymatous cells assume a yellow to dark reddish-brown coloration. This reaction is more particularly distinctive of tannin, and has the advantage of producing a precipitate which cannot diffuse from the cells in which it is produced.

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[1] A list of these has been given by Braemer, *Les Tannoïdes*, p. 125 (1890).

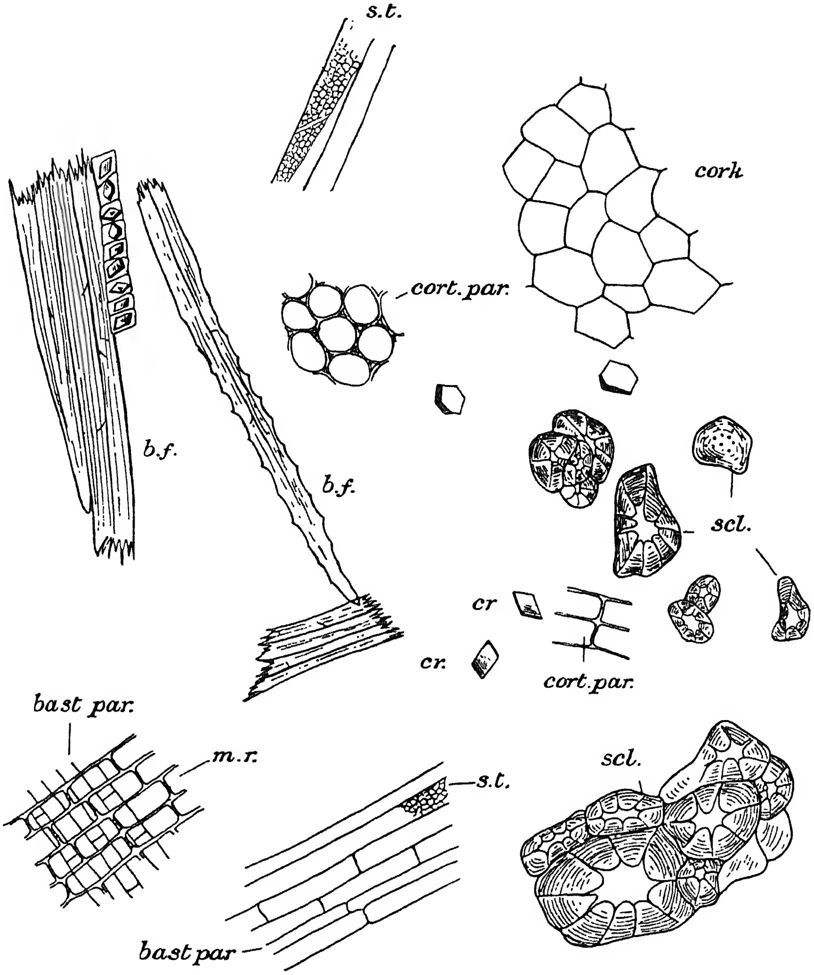

FIG. 94.—Powdered Witchhazel Bark. *b.f.*, fragments of bast fibres, with and without crystal cells adhering; *bast par.*, bast parenchyma; *cork*, cork cells in surface view; *cort. par.*, cortical parenchyma, in transverse and longitudinal section; *cr.*, crystals of calcium oxalate; *m.r.*, medullary ray, in radial section; *scl.*, sclerenchymatous cells, isolated and grouped; *s.t.*, sieve tubes, more or less broken. ×210.

Next examine tangential and radial sections, and identify in them the elements that have been observed in the transverse section. The sieve tubes are in these sections, as in the transverse, not so conspicuous as they were in cascara, but they may be found by careful searching.

For the isolation of the bundles of bast fibres, employ digestion with solution of potash, and, for the separation of the bast fibres from one another, maceration with potassium chlorate and nitric acid.

Examination of the Powder.—Proceed next to examine the powder as follows:

1. Mount a little in water or dilute glycerin. There is no conspicuous colour; the bast fibres and sclerenchymatous cells are nearly colourless, but some of the latter contain a reddish-brown colouring matter, as do also many of the parenchymatous cells and some of the cork cells.

2. Irrigate the preparation gently with solution of potash; there is no striking change of colour, but the bast fibres and sclerenchymatous cells acquire a yellowish tinge.

3. Mount a little in chloral hydrate (after alcohol): in this preparation the sclerenchymatous cells are conspicuous by reason of their number. Some are isolated, but the majority are in groups of varying size; the cells themselves also exhibit great diversity in size, shape, and the extent to which they have been thickened.

Bast fibres are also conspicuous; they are nearly colourless and accompanied by an abundance of calcium oxalate crystals; the cavities are very narrow.

Fragments of parenchymatous cells are abundant; among these comparatively large, well-defined, prismatic crystals of calcium oxalate can be detected, but no rosettes. Beaded parenchymatous walls are not numerous, and comparatively few have large pits.

The medullary ray cells are easily identified; they exhibit no unusual features.

The sieve tubes are inconspicuous; they seldom attain the size that is common in cascara bark (see fig. 94).

4. Treat some of the powder with potassium chlorate and nitric acid as directed for quassia wood; the sclerenchymatous cells and the bast fibres may be isolated, and their exact size and shape determined (but care must be taken not to prolong unduly the action of the oxidising mixture).

Cinnamon Bark

Source.—The bark of *Cinnamomum zeylanicum,* Breyn., freed from the epidermis and most of the cortex.

Preparation and Examination.—From a stick of cinnamon separate one or two of the outer pieces of the thin bark; soak them for twelve hours in water, or expose them for two days to a moist atmosphere; fix three or four together between pith; cut them all together, and transfer the sections to a small dish. Then fix the pieces so as to cut radial sections, and transfer these to another dish. Should the sections curl inconveniently they may be left in water for twenty-four hours or they may be straightened by the careful use of the dissecting needles.

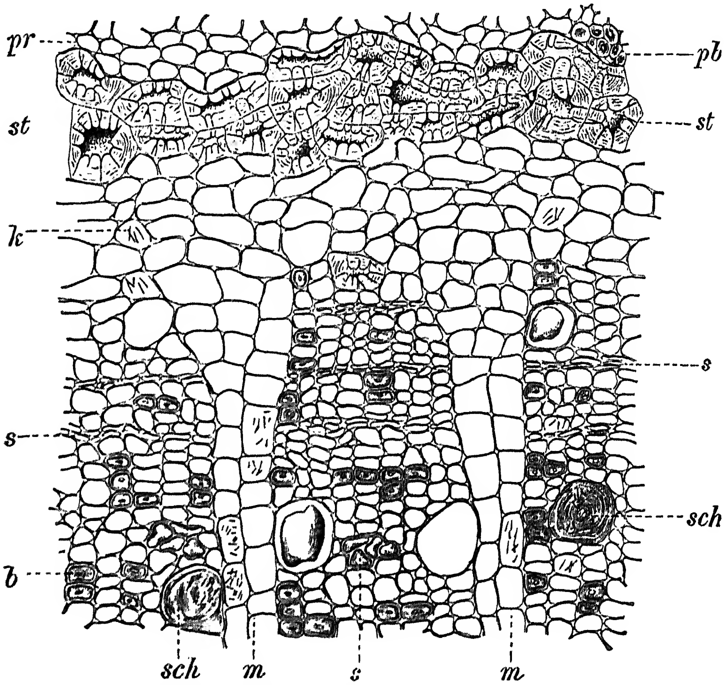

FIG. 95.—Cinnamon Bark, transverse section. *b*, bast fibres; *k*, crystals of calcium oxalate; *m*, medullary rays; *pb*, primary bast fibres (pericyclic fibres); *pr*, cortical parenchyma; *s*, sieve tubes; *sch*, secretion cells; *st*, sclerenchymatous cells, forming an uninterrupted ring. × 160. (Moeller.)

Examine a transverse section in chloral hydrate. Most of the cells of the bark are coloured dark brown.

Decolourise some sections by placing them in recently prepared solution of chlorinated soda for a few minutes; as soon as they are decolourised, transfer them to water. Mount one in solution of chloral hydrate, and examine.

The outermost tissue is (with the exception of fragmentary parenchymatous cells) a band of sclerenchyma, within which is the narrow bast ring traversed by medullary rays. There is no epidermis or cork, and traces only of primary cortex.

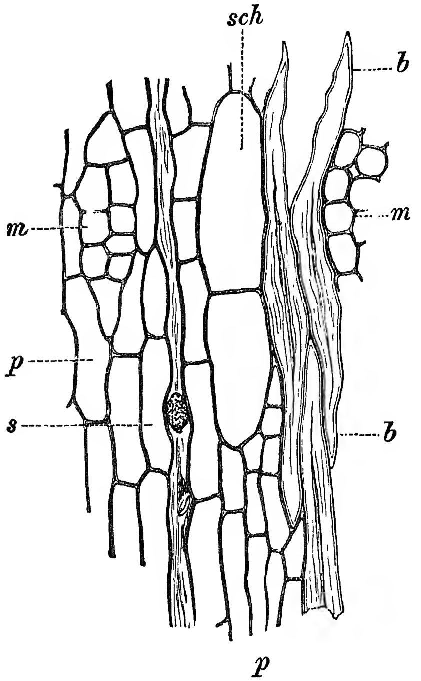

FIG. 96.—Cinnamon Bark, tangential section. *b*, bast fibres; *m*, medullary ray; *p*, bast parenchyma; *s*, sieve tube; *sch*, secretion cells. × 160. (Moeller.)

Examine the sclerenchymatous ring; the cells of which it is composed are mostly tangentially elongated (in transverse section), but some are nearly round; they may attain 150 μ or more in length and may contain starch grains. They have the characters that such cells usually possess, but are distinguished by the fact that the inner walls of many of them are thicker than the outer; frequently, however, this thinner wall is presented to the observer and can only be seen after the cells have been separated from one another (maceration with potassium chlorate and nitric acid) and made to roll. Search also in this ring for groups of fibres that may easily be recognised by their small size, complete thickening, and absence of pits; they are usually on the outer margin of the ring. These are groups of pericyclic fibres (primary bast fibres).

Examine the bast ring. The parenchymatous tissue abutting upon the sclerenchyma consists of tangentially elongated cells. The medullary rays are mostly two cells wide, and the cells are nearly isodiametric. The bast rays contain conspicuous bast fibres, rounded or four-sided in section; they are isolated or in small, often tangentially arranged groups of three or four; the walls are very thick, the lumen being reduced to a point. In the bast rays observe also the secretion cells. They are often empty; they may be distinguished from the bast parenchyma by their larger size, and may be made more conspicuous by warming a section in Soudan red, by which the walls are coloured red.

The sieve tubes can be recognised by the characters of the walls; towards the sclerenchymatous ring they often collapse into strands, in which the cell cavities are scarcely visible.

Mount a bleached section in corallin soda—many of the sieve tubes will show rounded masses of callus stained pink.

Notice in some of the cells of the medullary rays and bast parenchyma numerous minute, prismatic crystals; they are calcium oxalate, and will appear brilliantly illuminated when examined by polarised light. Among other cell contents to be examined note the starch grains.

Mount a section in diluted solution of iodine in potassium iodide; observe the abundance of starch grains which also occur in some of the sclerenchymatous cells.

Make a diagrammatic sketch of the transverse section, and enlarged sketches of a portion of the sclerenchymatous ring and of the secondary bast. Sketch a few starch grains also.

Treat radial sections as directed for transverse.

The secretion cells are easily seen; they are axially elongated; some are empty, but some contain yellowish volatile oil (or resin), or a mixture of this with mucilage, or mucilage alone. Mucilage is best seen in a section cut from bark that has not been soaked in water. Mount the section in dilute glycerin and examine; the mucilage slowly swells.

The sieve plates are small, transversely or slightly obliquely situated, and very numerous. The sclerenchymatous cells are nearly isodiametric; the parenchymatous cells next them are rather thick-walled and isodiametric, or slightly axially elongated. The bast parenchyma cells have thinner walls; they are strongly axially elongated, and exhibit large circular pits. The cells of the medullary rays are larger, squarer, sometimes but not always radially elongated, and have very thin walls; they can therefore be easily identified.

Treatment by Maceration, &c.—Digest a few fragments of the bark in 5 per cent. solution of potash in a water-bath for fifteen minutes; wash with distilled water, and tease out with the needles on a slide. The bast fibres can by this means be easily isolated, since they often occur singly. Examine and sketch one or two, preserving accurately the relative length and thickness.

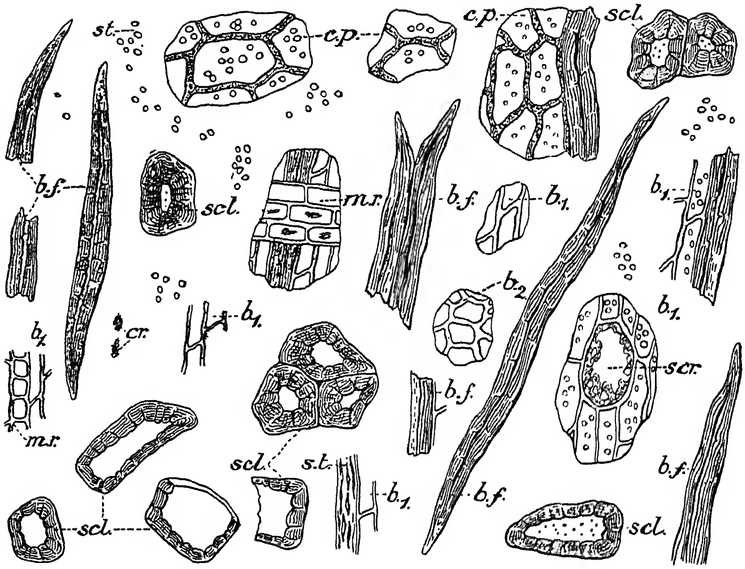

FIG. 97.—Powdered Cinnamon Bark. $b._1$, $b._2$, bast parenchyma in longitudinal and in transverse section; *b.f.*, bast fibres; *c.p.*, cortical parenchyma; *cr.*, crystals of calcium oxalate; *m.r.* medullary ray; *s.t.*, collapsed sieve tissue; *scl.*, sclerenchymatous cells; *scr.*, secretion cells; *st.*, starch. (Partly after Greenish and Collin.) ×150.

In this preparation numerous secretion cells containing droplets of yellowish oil or brown resin will be found. Sketch one or two.

From a small portion wash out the potash with distilled water; mount in corallin soda; the callus plates stain pink, but not as well as in the section bleached with chlorinated soda.

Macerate a few pieces of the bark with potassium chlorate and nitric acid, as directed for quassia wood; isolate, and examine the sclerenchymatous cells; observe the frequent one-sided thickening; as only one wall is thin, it is necessary to make the cells roll so as to present varying aspects. Sketch three or four cells.

Examination of the Powder.—Reduce some picked cinnamon to powder, and sift it through a No. 60 or No. 80 sieve.

Mount a little in water or dilute glycerin; examine the starch. The grains are small, often two, three, or four together. Sketch a few, preserving accurately the relative size.

Examine again after two hours or more. Note the colour of the fragments, varying from yellowish in the smallest to brown in the largest; the bast fibres and sclerenchymatous cells are colourless or yellowish, though they may sometimes appear coloured from the adhering walls of parenchymatous cells.

Mix 0·2 gramme of the powder with 10 c.c. of solution of chlorinated soda. Shake occasionally until the brown colour is changed to yellowish. Separate the powder by allowing it to settle, or by the use of the centrifuge. Wash with distilled water, and separate again. Pour off the supernatant liquid, and treat the residue as follows:

(1) Mix a little of the deposit with glycerin and examine. Observe in this preparation the bast fibres, starch, calcium oxalate, and cellular elements. The sieve tubes and secretion cells are not easily seen.

(2) Transfer a little to a slide, remove most of the water with filter paper, add a little Soudan red in glycerin, and warm gently to the boiling-point; let the slide stand for five or ten minutes, and examine. The secretion cells will be readily distinguished by the red colour which their suberised walls will have assumed. Others of the cell walls also frequently take a faint colour, but not nearly so deep as those of the secretion cells. Droplets of oil in the preparation are also stained deep red.

(3) Stain a little with corallin soda; the callus plates stain pink, rendering the sieve tubes easy of identification. Compare them with the section.

(4) Warm a little of the deposit with water or chloral hydrate. The starch will be gelatinised, and the

minute, prismatic crystals of calcium oxalate can easily be seen, both in the cells and scattered over the field. By polarising they can be made particularly conspicuous. In this preparation the characteristic pits on the walls of the parenchymatous cells can also be seen.

(5) From a little of the deposit remove the water and add a drop of strong hydrochloric acid; cover, and allow it to stand five or ten minutes. Then remove the acid, and add a drop of glycerin. The bast fibres and sclerenchymatous cells are now particularly well seen, and can be minutely examined.

Coarse powder may be separated by sifting as described for senna, the fine powder being examined as above, while from the coarsest fragments sections should be prepared. Part also of the coarse portion should be reduced to fine powder, and compared with that separated by sifting.

Fine Powder of Commerce.—This may be examined in the same way. Abundant fragments of parenchymatous cells, often difficult to identify, are present; many of the sclerenchymatous cells and bast fibres are broken; liberated starch grains are abundant.

The presence of vessels and wood fibres indicates probable adulteration with bark refuse; gelatinised starch grains would point to adulteration with the residue left after distillation of the volatile oil, although intact starch does not necessarily exclude such exhausted bark, as the starch grains of cinnamon are not readily gelatinised. Adulteration with bark that has been exhausted by percolation is best effected by chemical means.

Diagnostic Characters.—The chief diagnostic characters of powdered cinnamon are:

(*a*) The pervading yellowish to brown colour;
(*b*) The characteristic, slender, isolated bast fibres without crystal cells;
(*c*) The sclerenchymatous cells, frequently exhibiting one wall thinner than the others;
(*d*) The numerous small, often compound starch grains;
(*e*) The minute crystals of calcium oxalate;
(*f*) The secretion cells;
(*g*) The parenchymatous cells with characteristic pits.

Cassia Bark

Source.—The bark of *Cinnamomum Cassia,* Blume.

Examination.—Cassia bark closely resembles cinnamon bark in structure and may be examined in the same way. The transverse section often exhibits a layer of cork in which rows of cells with thickened walls alternate with cells with thin walls. The cells of the sclerenchymatous ring have thinner walls than the corresponding cells of cinnamon and exhibit a more conspicuous one-sided thickening; many contain small starch grains. The cells of the cortical parenchyma are also

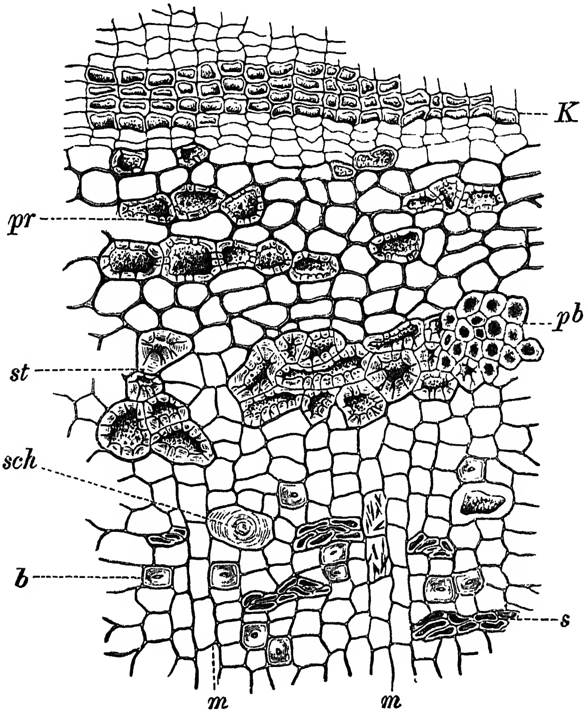

FIG. 98.—Cassia Bark, transverse section. *b*, bast fibres; *K*, sclerenchymatous cork cells; *m*, medullary rays; *pb*, primary bast fibres (pericyclic fibres); *pr*, cortical parenchyma, with sclerenchymatous cells; *s*, sieve tubes; *sch*, secretion cell; *st*, sclerenchymatous cells, forming an interrupted ring. × 160. (Moeller.)

frequently thickened on one side. The bast fibres are thicker than those of cinnamon, as are also the walls of the parenchymatous cells. The starch grains are rather larger than those of cinnamon.

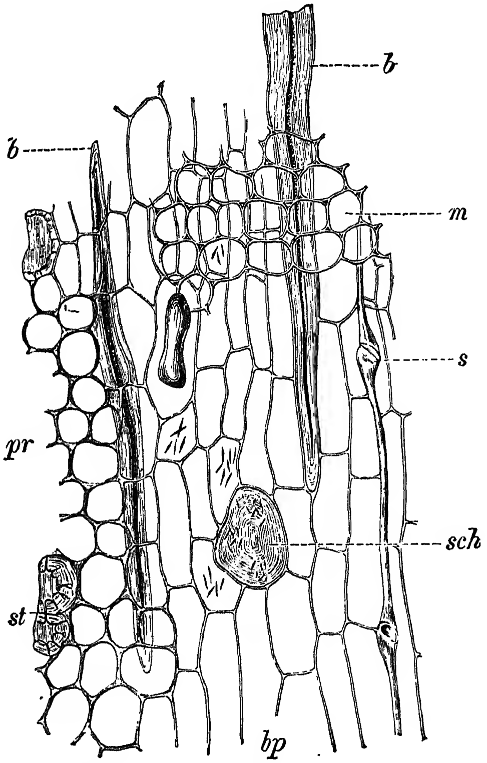

FIG. 99.—Cassia Bark, radial section. *b*, bast fibres; *bp*, bast parenchyma; *m*, medullary ray; *pr*, cortical parenchyma; *s*, sieve tube; *sch*, secretion cell; *st*, sclerenchymatous cells. ×160. (Moeller.)

The distinction of powdered cinnamon from powdered cassia is not difficult if fine grades of cinnamon are compared with typical samples of cassia. But the differences, which are themselves not great, diminish as one compares the lower grades of genuine cinnamon with the finest grades of cassia. In such cases the powders are extremely difficult to distinguish. The bark of *C. Burmanni* de Candolle, which is occasionally met with under the name of *Cassia vera*, is distinguished by the thickened, coarsely pitted cells of the medullary rays and also by the abundant tabular crystals of calcium oxalate. The bark of

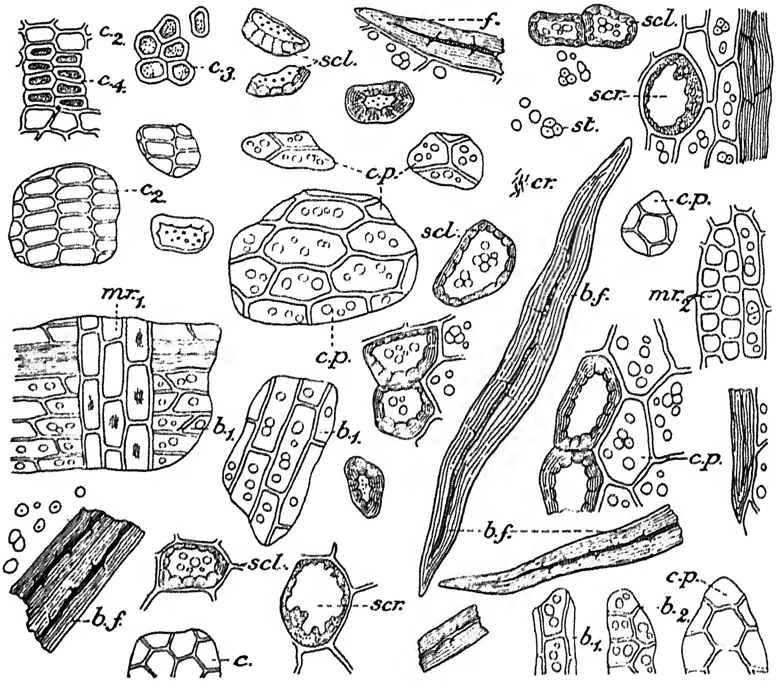

FIG. 100.—Powdered Cassia Bark. $b._1$, $b._2$, bast parenchyma in surface view and in section; *b.f.*, bast fibres; $c._1$, $c._2$, cork in surface view and in section; $c._3$, $c._4$, thick-walled cork in surface view and in section; *c.p.*, cortical parenchyma; *cr.*, crystals of calcium oxalate; $mr._1$, $mr._2$, medullary ray in radial and in tangential section, with crystals of calcium oxalate; *scl.*, sclerenchymatous cells; *scr.*, secretion cells; *st.*, starch. (Partly after Greenish and Collin.) ×150.

C. Tamala, Nees et Eberm., has similar crystals of calcium oxalate, but no such pitted cells.

Red Cinchona Bark

The bark of *Cinchona succirubra*, Pav.

Preparation and Examination of Sections.—For examination choose a rather thin, young, cultivated bark in quills; prepare it by soaking in water or dilute glycerin for twelve hours; cut transverse sections, and treat them in the usual way. Mount one in water. The section does not expand well in water; add a drop of solution of potash ; the colour deepens, and the section expands. Solution of caustic potash is frequently preferable to chloral hydrate, especially for hard barks and for such as contain much colouring matter resulting from the decomposition of tannin. Allow a few sections to stand in dilute glycerin for subsequent examination of the cell contents (see p. 183).

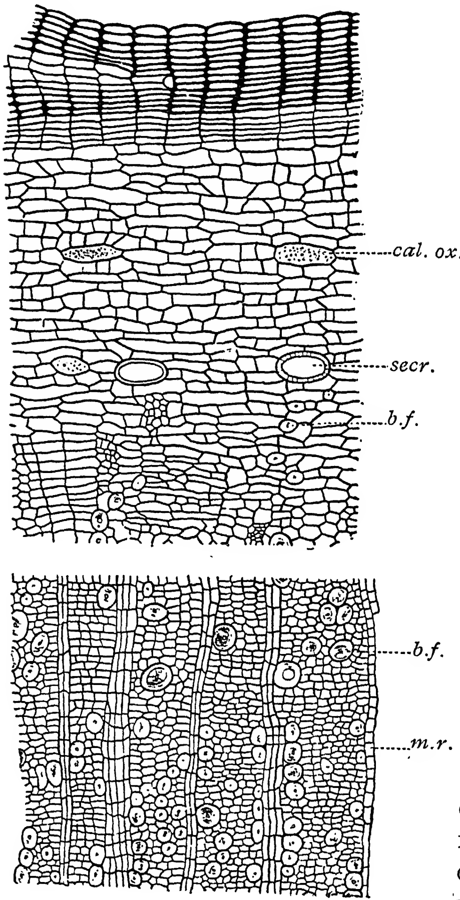

FIG. 101.—Cinchona Succirubra, transverse section of bark. *cal. ox.*, sandy crystals of calcium oxalate ; *b.f.*, bast fibres ; *m.r.*, medullary rays ; *secr.*, secretion tubes. (After Tschirch.)

Observe the cork cells ; they are very narrow, flattened, and contain a deep reddish-brown amorphous substance, even after treatment with caustic potash. Next to the cork there is often a phelloderm of varying thickness ; the cells are very much less coloured than those of the cork or primary cortex.

The primary cortex in young barks is often of considerable width ; its inner limit is not very well defined, but on examination large, isolated, usually empty cells may be discerned near the commencement of the bast rays ; they form a diffuse ring near the junction of the primary cortex with the bast ring. When young they contain a substance of gum-resinous nature, but in commercial barks they are often empty. Careful examination of transverse and longitudinal sections, after expansion of the walls by solution of potash, show these cells to be strongly axially elongated secretion tubes lined with delicate secreting cells which, in the dry drug, are collapsed.

The walls of the cells of the primary cortex are encrusted with a reddish-brown amorphous substance and are collenchymatous at the angles. Some of the cells are filled with sandy crystals of calcium oxalate; others contain small simple starch grains or compound grains with two or three components.

The medullary rays are one to three cells wide, and exhibit no striking features.

The bast rays are made up of bast parenchyma, sieve tubes and bast fibres, but the colour of the parenchyma is so dark that but few details can be seen; the cells contain calcium oxalate and starch.

The bast fibres, on the other hand, are very conspicuous by reason of their size. They are isolated, and arranged in irregular radial rows. Many measure from 40 to 60 μ, some even 90 μ in diameter. The lumen is often reduced to a point; the wall is colourless, very distinctly striated, and traversed by rather numerous pores. These bast fibres are not accompanied by crystals.

Remove the colour by bleaching the sections with chlorinated soda. Wash them once or twice in distilled water, and mount one in water.

The walls of all the cells can now be much more distinctly seen; those of the cortical parenchyma often exhibit large pores, but in the bast rays even now the sieve tubes can with difficulty be distinguished from the bast parenchyma.

Stain a bleached section with corallin soda: the callus plates stain, and the sieve tubes can thus be identified. Collapsed sieve tubes occur in the outer portion of the bast.

Examine a section after prolonged maceration in dilute glycerin; observe occasional (sometimes numerous) small starch grains and the abundant, reddish-brown, amorphous contents of the parenchymatous cells; the bast fibres appear in section colourless or nearly so.

Treat radial sections in the same way. Observe particularly the bast fibres; they are spindle-shaped; the wall is distinctly striated, the lumen is narrow, and the pits widen towards the lumen. The sieve tubes are small. The principal sieve plates are placed transversely or nearly so, but there are very small ones in vertical rows on the tangential walls.

Treatment by Maceration.—Digest some pieces of cinchona bark for about fifteen to thirty minutes in 3 per cent. solution of potash, and tease out.

The bast fibres are yellow; they are completely isolated, and their shape can be well seen. Observe numerous parenchymatous cells filled with prismatic crystals. These crystals are the precipitated alkaloids, and dissolve readily in dilute acid.

Stain the callus plates of the sieve tubes with corallin soda, bleaching first with chlorinated soda as directed for cinnamon bark, if necessary.

Examination of the Powder.—Examine powdered red cinchona bark as follows:

(1) In water. The bast fibres are mostly isolated; some are intact, some broken, but even the broken fibres are easily recognised by their large size and characteristic pits; some are almost colourless, others yellowish-brown; the walls of adhering parenchymatous cells may make them appear darker than they really are.

There are also very numerous fragments of yellowish to deep reddish-brown parenchymatous tissue.

(2) In dilute glycerin, allowing the preparation to stand. For the sake of uniformity the colour should be judged on this preparation under a magnification of 200 diameters. The parenchymatous cells have thin or moderately thick walls.

(3) In chloral. Warm in the water-bath for a few minutes, or allow it to stand several hours. The bast fibres become very distinct; the parenchymatous cells lose much of their colour, and can be identified. The cork is composed of polygonal cells, the colour of which is usually very dark brown.

(4) In chloral iodine, for the detection of starch.

(5) After bleaching with chlorinated soda and staining with corallin soda, for the detection of the sieve tubes.

Diagnostic Characters.—

(*a*) The remarkable bast fibres, with their characteristic walls and pits.

(*b*) Absence of other sclerenchymatous elements.

(*c*) The yellowish- or reddish-brown colour of the parenchyma.

Identification of an Unknown Powder as that of a Bark

In the examination of a powder of unknown origin it may be necessary to identify it as that of a powdered bark. There is only one characteristic element that is present in all bark powders, and upon which therefore particular stress must be laid; this is the sieve tube. Its presence necessarily indicates the presence of bast tissue, and, as barks are composed more or less largely of bast, the presence of numerous and comparatively large sieve tubes is strong presumptive evidence that the powder is derived from a bark. Cork tissue is also present in most powdered barks, but not necessarily, since it is sometimes removed during the preparation for the market. Nevertheless, the occurrence of much cork tissue is strong confirmatory evidence, as is also that of bast fibres and sclerenchymatous cells.

All these elements may, however, be present in the bark that is attached to, and constitutes a portion of such organs as roots, rhizomes, &c. It is certainly true that sclerenchymatous cells and fibres are not usually present in roots or rhizomes, but to this there are many exceptions.

A powdered bark should be free from vessels, but fragments of wood may occasionally be found adhering to barks and constitute a source of contamination. Much vascular tissue would therefore indicate the presence of wood. Chlorophyll and the tissue in which it is chiefly found (palisade and spongy parenchyma) should also be absent, as well as epidermis; the presence of these would indicate admixture of leaf powder. Aleurone grains and fixed oil, characteristic reserve materials of seeds, should also be absent.

Having determined that the powder is derived from a bark and is free from contamination with powder derived from other organs, the endeavour may be made to establish its identity.

As is the case with leaves, this is at present a matter of considerable difficulty and requires great experience. Much information may be obtained from the published descriptions of the anatomy of the more important and more common barks.

SECTION X

SEEDS

INTRODUCTION

The student is strongly recommended to study carefully in his text-book of botany the structure of the seed; the object of the following brief account is to direct his attention to those parts of the seed which usually afford valuable diagnostic characters.

The fully developed seed consists commonly of two seed coats, an outer and an inner, enclosing a kernel. Sometimes, however, only one seed coat is present, while occasionally there are three, the third being in the shape of an arillus or arillode. Now and then a caruncule is attached to the seed.

The kernel of the seed may consist simply of the embryo, or of the embryo accompanied by either endosperm or perisperm, or both.

In those seeds in which the kernel consists of the embryo only, in which, therefore, the tissues of the nucellus and embryo sac have not developed into perisperm and endosperm respectively, the remains of the younger stages of these two tissues are usually to be found. As the contents of their cells have been utilised by the embryo for its development, the empty cells become pressed together, and ultimately form delicate hyaline membranes, the structure of which is perceptible in surface view only. Difference of opinion exists as to whether these membranous layers are to be reckoned as part of the seed coat or part of the kernel. Seeing that they afford an additional, if only very slight protection to the embryo, they may be included with the seed coats, which, therefore, will embrace all the tissues of the seed exterior to the kernel.

The following parts may, therefore, be present in a seed, and each of them may furnish valuable diagnostic characters:

1. Caruncule.
2. Arillus.
3. Outer seed coat.
4. Inner seed coat.
5. Perisperm.
6. Endosperm.
7. Embryo.

The seed coats exhibit an infinite variety of structure, but the following tissues may be mentioned as of frequent occurrence:

1. *Mucilaginous Layer.*—Many seeds, especially dicotyledonous seeds, contain a layer of cells in which a quantity of mucilage has been secreted. As this mucilage is often destined to attach the seeds to the soil upon which they fall, it is usually the epidermal layer that is mucilaginous. Seeds that are thus provided with a mucilaginous epidermis surround themselves with a layer of mucilage when they are soaked in water (mustard seed, linseed, quince seed).

2. *Sclerenchymatous Layer.*—Very frequently one or more rows of cells in the seed coats develop into a layer of sclerenchymatous cells or fibres, the object of which is to supply the seed coat with the necessary firmness and resisting power. This layer is often developed from the inner epidermis of the outer seed coat (mustard, linseed), though this is by no means necessarily the case; sometimes it is developed from the outer epidermis of the outer seed coat (henbane). The cell walls are frequently coloured and thickened in a characteristic manner; hence they possess a high diagnostic value.

3. *Pigment Layer.*—The particular colour of the seed coat is often due to colouring matter deposited in a single (or occasionally multiple) layer of cells (linseed, black mustard seed).

In other cases certain layers of the seed coats develop in particular ways; thus, in cardamom seeds, in addition to a sclerenchymatous layer, there is a single layer of large rectangular cells in each of which volatile oil is secreted. In nux vomica, the epidermal cells of the outer integument develop into remarkable hairs, while all the remaining layers become obliterated; in areca seeds, sclerenchymatous cells of varying shapes are developed, together with much tannin.

The tissues derived from the outer and inner integuments of the ovule may be followed by obliterated layers derived from the nucellus or from the embryo sac, as well as by the proteid layer. The latter is usually the epidermis of the endosperm, and is generally well preserved, the cells being moderately thick-walled and filled with proteid matter and oil. The obliterated layers of the nucellus and embryo sac often form hyaline membranes in which little structure is discernible, at least in transverse sections.

The kernel of the seed may consist of the embryo alone, or of the embryo and endosperm, or of the embryo, endosperm and perisperm.

In structure the perisperm and endosperm are usually very similar. The cells of which they consist may have very thin walls, in which case the contents form the reserve material, or the walls may be thickened, sometimes to such an extent as almost to obliterate the cavity, the thickening being reserve cellulose or mucilage, or some modified form of cellulose (areca nut, foenugreek seed, nux vomica).

In seeds that contain no endosperm, the embryo itself usually fills with reserve material.

Very important for the present purpose is the determination of the thickness and nature of the cell walls of these tissues, as well as the character of the pits and the nature of the reserve material.

The infinite variety exhibited by the layers of the seed coats, both as regards the cells of which they are composed and the contents of those cells, renders the seeds very interesting to examine, and often easy to identify, in either the entire or the powdered state. Diagnostic characters are to be looked for particularly in the following cells and cell contents:

(*a*) The epidermis of the seed coat.
(*b*) The sclerenchymatous layer.
(*c*) The pigment layer.
(*d*) The cell walls of the kernel.
(*e*) The reserve material of the kernel.

In addition to these, valuable diagnostic characters may be found occasionally in others of the layers present.

Aleurone Grains

In a preceding chapter the examination of starch grains was dealt with, and it was pointed out that they constitute a most important means of identifying certain foods and drugs by reason of the varying characters that they exhibit. These characters are fairly constant for the starch grains of one and the same drug, but they vary in the grains of different drugs. Moreover, it is obvious that the presence of starch grains in a powdered drug which normally contains no starch indicates either substitution or adulteration, or possibly, as in the case of seeds, the substitution of the unripe for the ripe organ.

Starch is one of the commonest of the reserve materials of seeds. It is accompanied by proteid matter, which usually takes the form of minute rounded grains. In the pea, for example, the interspaces between the starch grains are packed with these minute grains of proteid matter embedded in a granular ground substance. They can be readily seen if a very thin section from a dry pea be examined in glycerin to which a little solution of iodine in potassium iodide has been added, as under such conditions they acquire a yellowish-brown colour (fig. 102). To these grains the name of *aleurone grains* or *proteid grains* has been given.

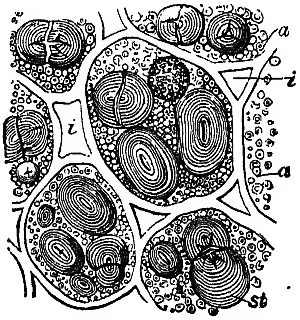

FIG. 102.—Section of the Cotyledon of Pea. *a*, *a*, aleurone grains; *i*, *i*, intercellular spaces; *st*, starch. Magnified. (After Sachs.)

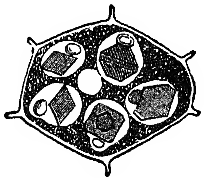

FIG. 103.—Cell from the Endosperm of Castor Seed, showing large transparent aleurone grains with globoids and crystalloids embedded in them. (After Sachs.)

In many seeds, especially in such as contain oil or fat as the chief reserve material, the aleurone grains attain a much larger size than they do in starchy seeds; they then occur immersed in the oil or oily plasma that fills the cells (fig. 103).

Like starch grains, the aleurone grains of one and the same drug exhibit a remarkable constancy in their characters, but they often differ in size, shape, or composition from the aleurone grains of other drugs. Here, then, is a means by which it is possible, within certain limits, to distinguish one seed from another. This becomes especially important when the seed coats, in which the most valuable diagnostic characters reside, have been removed, and the chief means of identification thus destroyed. Such is the case with the flour prepared from decorticated seeds, or the very finely sifted flour from ordinary seeds, for, in both cases, but very few fragments of the seed coats are to be found; the cells of the endosperm or cotyledons seldom exhibit any marked differences in the cell walls or cell contents, excepting the aleurone grains. Since aleurone grains are found in ripe seeds only, it follows that their presence in a powder indicates the presence of a powdered seed or part of a seed.

The following table[1] gives the sizes and contents of the aleurone grains of some of the common oil seeds; small grains (1 μ to 2 μ) are often also present:

	Size.	Globoids.	Crystalloids.
Papaver somniferum .	3 μ to 7 μ	numerous small	one
Ricinus communis .	7 μ to 15 μ	one to several	one or two
Sesamum indicum .	10 μ to 12 μ		
„ „ .	2 μ to 9 μ	many small	none
Cannabis sativa . .	4 μ to 8 μ	one	one
Brassica sinapioides .	7 μ to 16 μ	numerous small	none
„ Juncea . .			
„ Napus . .			
Amygdalus communis .	10 μ to 15 μ	—	one to three
„ „ .	3 μ to 8 μ	one to three	none
Linum usitatissimum .	10 μ to 19 μ	one	one to three
Cocos nucifera . .	25 μ to 56 μ	numerous small	one to many

It becomes very important, therefore for the microscopist to study especially (*a*) the means by which aleurone grains may be recognised, and (*b*) the means by which the aleurone grains of one seed may be distinguished from those of another. For this purpose, seeds containing fixed oil are preferable to those that contain starch, as their aleurone grains are larger and better developed.

The student should now make himself familiar with the principal characters and properties of these remarkable bodies.

The following constituent parts have been observed in aleurone grains, viz. *ground substance*, *crystalloid*, *globoid*, and

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[1] Tschirch.

calcium oxalate crystals. It is, however, comparatively rare to find all of them in one and the same grain.

Ground Substance.—The ground substance in which the crystalloid, globoid, &c., are embedded is quite amorphous and usually finely granular in appearance. It is generally readily soluble in water or, if not soluble in this medium, it is always more or less vigorously attacked by it. Hence water is not a suitable medium in which to examine aleurone grains. Fixed oils and glycerin, on the other hand, have no appreciable action upon it. Prolonged (eight days) maceration in alcohol renders the ground substance insoluble in water; therefore, the characters of the aleurone grains cannot be accurately determined in seeds or sections that have been subjected to such treatment; but a short maceration (twelve hours) in absolute alcohol is advantageous, inasmuch as it makes the grains more resistent to water, and dissolves the fixed oil, which usually accompanies them, so that the properties of the grains can be more easily studied.

In very dilute (0·3 per cent.) caustic potash the ground substance dissolves in all cases rapidly and completely. Lime water and dilute alkalies in general also dissolve it. It is also soluble in solutions of sodium chloride (1 to 10 per cent.), magnesium sulphate (1 to 20 per cent.), and disodium hydrogen phosphate (saturated solution).

Crystalloids.—The crystalloids consist, like the ground substance, of proteid matter, which, however, as the name indicates, has assumed a crystalline form; according to Schimper,[1] they belong either to the regular or to the hexagonal system. They differ from true crystals in the inconstancy shown by their angles, and in the fact that, under certain conditions, they swell. They may be so large as to constitute by far the bulk of the grain, or they may be embedded in a larger amount of ground substance.

They are less soluble than the ground substance of the aleurone grain. They are seldom soluble in water, or in saturated solution of disodium hydrogen phosphate, but always dissolve in dilute solution of potash.

Both ground substance and crystalloid are coloured brown by iodine solution, yellow by saturated solution of picric acid, and red by Millon's reagent.

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[1] *Zeitschr. f. Krystallographie*, 1880.

Globoids.—These bodies, which occur in many aleurone grains, are usually rounded or ovoid in shape. They consist, according to Pfeffer, of magnesium and calcium combined with phosphoric acid and with an undetermined organic acid. They are insoluble in water and in dilute caustic potash, but are soluble in dilute acids, in saturated solution of disodium hydrogen phosphate, and in solutions of various other salts. They do not colour with iodine or with picric acid, and the latter reagent may, under certain circumstances, dissolve them. They vary very much in size ($0{\cdot}5\ \mu$ to $10\ \mu$), but are generally small ($1\ \mu$ to $3\ \mu$). In those aleurone grains in which one or more crystalloids are accompanied by a globoid the latter is commonly situated at the narrower end of the grain (which is usually ovoid).

Calcium Oxalate.—This substance occurs most frequently in the shape of small rosettes, sometimes, but comparatively seldom, in single crystals, or in groups of a few acicular crystals. It is easily identified by the usual microchemical tests.

Aleurone grains exhibit a great diversity of size and shape. Many are rounded; this is especially the case with the very small ones; frequently they are ovoid or polygonal, or irregularly angular. Sometimes each cell contains a large grain accompanied by a number of small ones, or it may contain several of varying size, or a few or many of tolerably uniform size.

In their composition they also exhibit great variety. Very often the grain contains one globoid and from one to three crystalloids embedded in a rather scanty ground substance; or it may contain numerous minute globoids in a large quantity of ground substance.

The following experiments will serve to introduce the student to a suitable method for recognising and examining these bodies.

Take a castor seed (*Ricinus communis*); remove the seed coats, cut very thin transverse sections of the endosperm, and defat them by maceration for fifteen to thirty minutes in a mixture of ether and alcohol (equal parts), or, in this particular case, in absolute alcohol; transfer them to alcohol. Mount one in alcohol.

Observe, especially near the thin edge of the section, large ovoid or rounded bodies; they are aleurone grains, but they do not well exhibit their structure when examined in this medium.

Irrigate gently with iodine water, or dilute solution of iodo-potassium iodide; the crystalloid and globoid become visible; the former is coloured yellow; they are embedded in a ground substance, and appear surrounded by a delicate membrane. Irrigate with very dilute potash (0·3 per cent.); the membrane, ground substance, and crystalloid dissolve, the globoid remains undissolved.

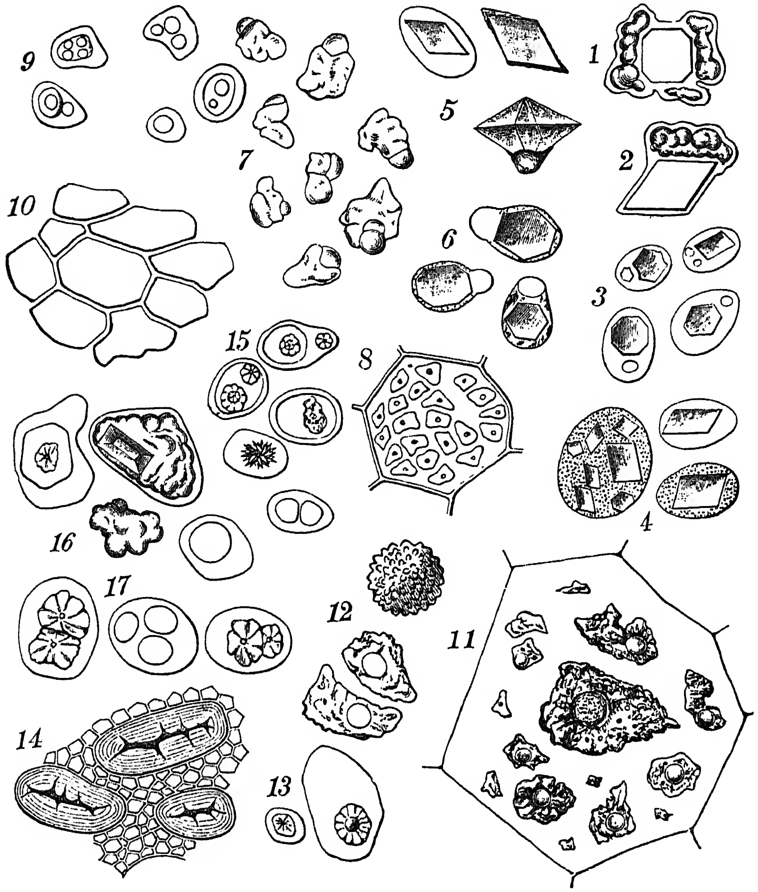


Fig. 104.—Aleurone Grains of various Seeds. 1 and 2, *Bertholletia excelsa*; 3, *Ricinus communis* (after treatment with water); 4, *Elaëis guineensis*; 5, *Myristica fragrans*; 6, *Cannabis sativa*; 7, *Datura Stramonium*; 8, *Gossypium, sp.*; 9 and 10, *Cydonia vulgaris*; 11, 12, 13, *Amygdalus communis*; 14, *Phaseolus vulgaris* (with starch grains); 15, *Coriandrum sativum*; 17, *Fœniculum, sp.* (Tschirch.)

Repeat this experiment, using a saturated aqueous solution of picric acid instead of iodine ; the aleurone grains are coloured bright yellow.

Mucilage

Substances of a mucilaginous or gummy nature are very frequently present in the parenchymatous cells of vegetable organs, and are liable to escape detection, as they are usually nearly colourless and exhibit but few distinctive reactions.

These substances, which may be included in the generic term ' mucilage,' occur in a number of varieties differing from one another in their mode of production as well as in their nature and composition. The varying degrees of solubility, the varying extents to which they swell, and the reactions they yield indicate that many mucilages are not homogeneous bodies, but mixtures in which sometimes one, sometimes another variety preponderates.

Mucilage may result from a transformation of the cell wall, as is the case with tragacanth, or it may be produced in the protoplasm and deposited on the whole or a portion of the surrounding cell wall in the form of a secondary thickening. The latter is the more common in drugs. Usually the mucilage is deposited in successive layers, and exhibits, therefore, either in the dry state or after suitable treatment, a more or less pronounced stratification. Not unfrequently these layers may be separated from the cell cavity by a delicate wall of cellulose, or even similar walls may separate the layers of mucilage from one another. Hence when such a mucilaginous layer, bounded by a cellular wall; is swollen by the addition of water, the delicate cellulose wall becomes visible and gives rise to the impression that the mucilage has been contained in a distinct cell. Such, however, is not the case.

All varieties of mucilage agree in being insoluble in alcohol and in glycerin, but, as already observed, they either swell or dissolve in water. In sections examined in glycerin or alcohol, the mucilage appears as transparent or granular thickenings of the cell wall, which, however, may be so considerable as to fill the cell cavity. Irrigated with water, the mucilage usually swells ; with alcohol, it again contracts ; with solution of subacetate of lead, it becomes yellowish and granular.

There is no general stain for mucilages, but the following reagents are the most useful.

(*a*) Solution of corallin in 25 per cent. solution of sodium carbonate.

(*b*) Solution of ruthenium red in 10 per cent. solution of lead acetate.

(*c*) Saturated aqueous solution of Bismarck brown.

(*d*) A suspension of Indian ink in water; in this case the mucilage, which does not absorb the black particles, is conspicuous as transparent, gelatinous masses.

White Mustard Seed

Source.—The seed of *Brassica alba*, Boiss.

Preparation and Examination of Sections.—Before proceeding to cut sections of the seed, the student should make himself familiar with its structure. Soak a few seeds in water; they will surround themselves with mucilage; when thoroughly softened, use the dissecting needles to strip the seed coats from one of them. Examine the kernel. It consists of two folded cotyledons, embracing the small radicle. Cut another seed in half, midway between the hilum and the apex; examine the cut surface with a lens; it exhibits sections of the cotyledons and radicle surrounded by the seed coats. There is no visible endosperm.

Prepare some seeds for section cutting as follows:

Mix a few with gum and glycerin (see list of reagents), put them on the flat end of a small cork, and let them dry on. The glycerin will prevent the gum from becoming brittle, but the mucilage will hold the seeds firmly enough for cutting. Or a single seed may be fixed in pith, with the hilum upwards, so that transverse sections may be obtained.

Cut a number of transverse sections as nearly through the centre of the seeds as possible. Keep them in alcohol.

Seed Coats.—The seed coats may separate from the kernel, but this is of no consequence. Transfer several sections of the seed coats to a slide; immerse them in water to dissolve the gum, and remove the watery solution with filter paper; mount in chloral hydrate.

For examination, select a section that has passed through the centre of the seed and exhibits sharply defined lines of cells without any blurring from outer layers overlapping inner layers, as would be the case with tangential sections. Observe the following layers:

(1) An epidermis (fig. 106, *E*), consisting of large, rectangular cells, flattened or sometimes nearly square in transverse section. They average from 40 to 80 μ in length and have very thin walls, the outer one being usually convex. Careful examination shows them to be filled with a transparent, colourless, striated substance, in the centre of which there is a narrow cavity.

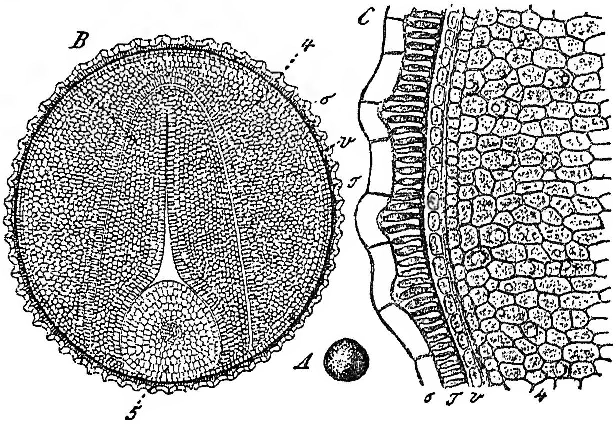


FIG. 105.—Mustard Seed (black.) *A*, entire seed, ×3; *B*, transverse section, ×65; 4, the cotyledon; 5, the radicle; *C*, portion of the same, still further enlarged; σ, the mucilaginous epidermis. (Berg.)

Mount a fresh section in alcohol (without treatment with water) and irrigate gently with water, watching the epidermis. The cells swell and become more distinct. Remove the water and add solution of ruthenium red; the substance contained in them stains bright pink. It is mucilage, which is deposited on the walls of the cells and swells in contact with water, often exuding in the form of dome-shaped, transparent masses from cells that have been cut by the razor.

(2) A layer, usually two cells thick, composed of large parenchymatous cells, the angles of which often exhibit intercellular spaces and the walls collenchymatous thickening (fig. 106, *Gr.*). They are empty and often collapsed, and hence not

easily seen, but here and there they are distinct, and may be made more so by warming the preparation with chlorzinciodine or strong (20 per cent.) solution of potash. They are very easily seen in surface sections.

(3) A single row of palisade cells (fig. 106, *Ps.*). These vary from 5 to 10 μ in width and from 30 to 40 μ in length. They are tolerably uniform in length, but nevertheless a slight increase is perceptible at regular intervals, corresponding to the undulating course of the inner walls of the parenchymatous layer above it.

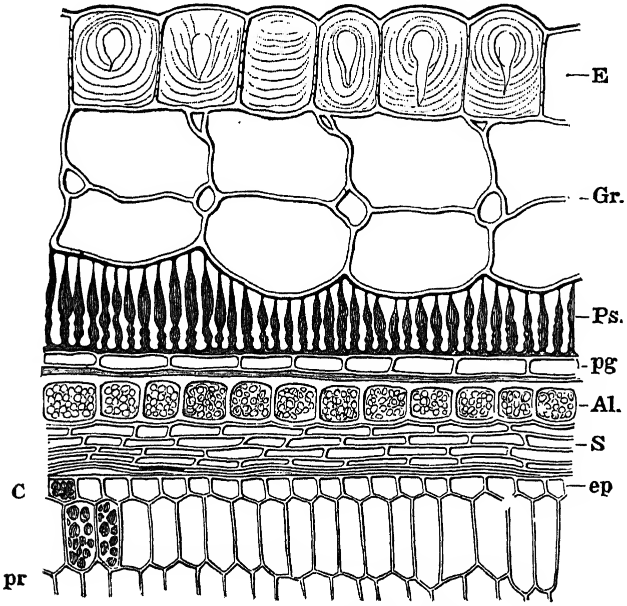


FIG. 106.—White Mustard Seed, transverse section. *Al.*, aleurone layer; *C*, cotyledon; *E*, mucilaginous epidermis of seed coats; *ep*, epidermis of cotyledon; *Gr.*, large subepidermal parenchymatous cells; *pg*, parenchymatous cells corresponding to the pigment cells of black mustard; *pr*, parenchyma of cotyledon; *Ps.*, sclerenchymatous palisade cells; *S*, collapsed cells of nucellus. (Vogl.)

The walls of the palisade cells are pale yellow in colour, the inner and radial being much thickened. This thickening is not uniform, but tapers away rather abruptly in the upper part of the cell, the wall becoming thin, wavy, and often collapsed. The lower thickened part has usually an irregular, jagged edge. The cell wall is but slightly lignified.

(4) A thin colourless membrane which is composed of two or three rows of collapsed parenchymatous cells. No cell contents are visible, and, indeed little structure can be made out beyond indications of cell lumina (fig. 106, *pg.*). One layer of cells can be made more distinct by caustic potash.

(5) A single row of very conspicuous rectangular cells with rather thick walls (fig. 106, *Al.*). These cells contain oil and aleurone grains, which can be stained by appropriate reagents; hence the layer is termed the aleurone or proteid layer. In the section under examination the contents will have been altered by the action of the water and chloral hydrate with which the sections have been treated. The cells of the aleurone layer vary from 15 to 30 μ in width, and are about 15 μ in height.

(6) Lastly, there is a colourless hyaline layer composed of several rows of more or less collapsed parenchymatous cells (fig. 106, *S*). These may be made more distinct by the use of concentrated solution of potash or other reagents that induce a swelling of the cell wall; they generally exhibit rather thin walls, and long narrow cavities without any perceptible contents.

The layers 1, 2, and 3 are probably derived from the integuments of the ovule, while 4, 5, and 6 represent the remains of the nucellus and endosperm.

Kernel.—Fix a seed in pith with the hilum upwards, and cut sections, as thin as possible, through the centre; they will cut the radicle transversely.

Mount one in chloral hydrate; observe the numerous globules of oil that exude.

Transfer the remainder to ether or a mixture of ether and alcohol in a small corked tube, and macerate for a few minutes to remove the oil; then pour off the ether, add a little alcohol, and transfer to a dish.

Transfer a few of the defatted sections to a drop of water on a slide, remove the water, and add chloral hydrate.

Examine first the sections of cotyledons. The tissue is thin-walled, and shows a distinct, small-celled epidermis on either side (fig. 108, *cot.*). Under the epidermis of the lower surface the cells are elongated to form a varying number of rows of palisade cells; under the upper epidermis the cells are more rounded. In the centre the procambial strands, formed of very small cells, can be seen.

The radicle exhibits an epidermis, next to which there is a cortical tissue of cells that have rather thicker walls than the cells of the cotyledons, and exhibit intercellular spaces. In the centre is the rudimentary stele.

Sketch a portion of cotyledon and radicle.

Next examine the cell contents. Mount one or two very thin, defatted sections in a saturated solution of picric acid; after five minutes examine the cells at the thin edges of the sections. Observe that they are filled with irregularly oval or rounded bodies that are stained yellow and appear granular. They are aleurone grains. They are often very conspicuous if the stained section is mounted in glycerin (fig. 108, *al.*). Sketch a few. Irrigate very gently with dilute solution of potash (0·3 per cent.); they rapidly dissolve, each leaving a number of minute granules, visible with difficulty, behind. These granules are globoids.

Mount another section direct in glycerin (without water); the aleurone grains are quite distinct; they will dissolve, as before, in diluted potash.

The aleurone grains can also be stained with other reagents, among which iodine is perhaps the best, as it distinguishes them from other substances, more particularly from starch grains. Iodine water, or diluted solution of iodopotassium iodide, may be used, and the grains subsequently dissolved by very dilute solution of potash.

Mount a section of the kernel in water and irrigate with solution of potash; the section acquires a yellow colour. This reaction is characteristic of mustard seed.

Mount another section in Millon's reagent; the cell contents gradually acquire a brick-red colour (reaction for proteid matter). Some of the cells which are larger than their neighbours are distinguished by the brighter colour they assume with Millon's reagent; these are said to contain the myrosin.

Disintegration of the Seed Coats.—Soak some seeds in water for a few hours, and also some others in solution of potash for an hour or more. Cut one of each open, and remove the kernel. Hold a piece of the seed coat firmly with a needle, and scrape the surface vigorously with the sharp edge of a glover's triangular needle; disintegrate it as much as possible by both cutting and scraping. Examine the *débris* thus obtained in solution of potash. Search for and identify the layers

1, 2, 3, 4, 5, and 6 that have been found in the transverse sections. They will now present the same appearance as they will in the powdered drug, and hence this examination is most important.

(1) Epidermis (fig. 107, *ep.*): the cells are easily identified

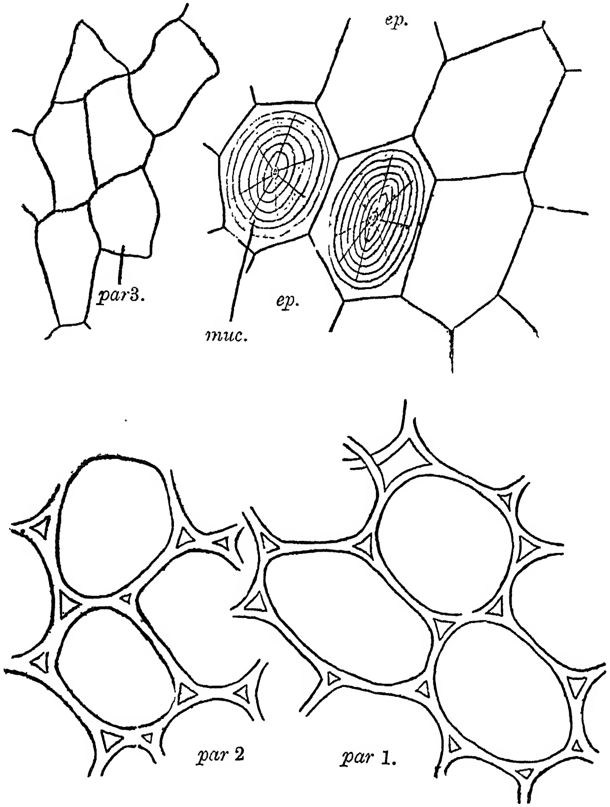


FIG. 107.—White Mustard Seed, from the powder. *ep.*, epidermis (in two cells the mucilage, *muc.*, is figured); *par* 1., the upper of the two layers of parenchyma; *par* 2., the lower layer; *par* 3., cells of the layer *pg* in fig. 106. ×240.

by their large size (45 to 60 μ in diameter), polygonal, transparent, mucilaginous contents, and thin walls. The mucilage exhibits circular striations and a small central cavity from which delicate lines frequently extend to the wall. It can be stained, if necessary, with solution of ruthenium red.

(2) This layer usually adheres to others, especially to No. 1:

it consists of large rounded cells with rather thick walls and intercellular spaces; the walls are often thickened near the angles. By focussing down, the presence of a second similar layer can often be determined (fig. 107, *par* 1., *par* 2.).

(3) The palisade cells present their surface view, which is quite different from the profile. The fragments of this layer are easily distinguished by their pale yellow colour. On examination they appear as small, polygonal cells with thick walls and small cavities. The thick walls are the thickened radial walls seen in the section (fig. 108, *scl.*). If the cells are lying with their outer surface upwards—that is, in the position in which they exist in the seed—then, on focussing carefully

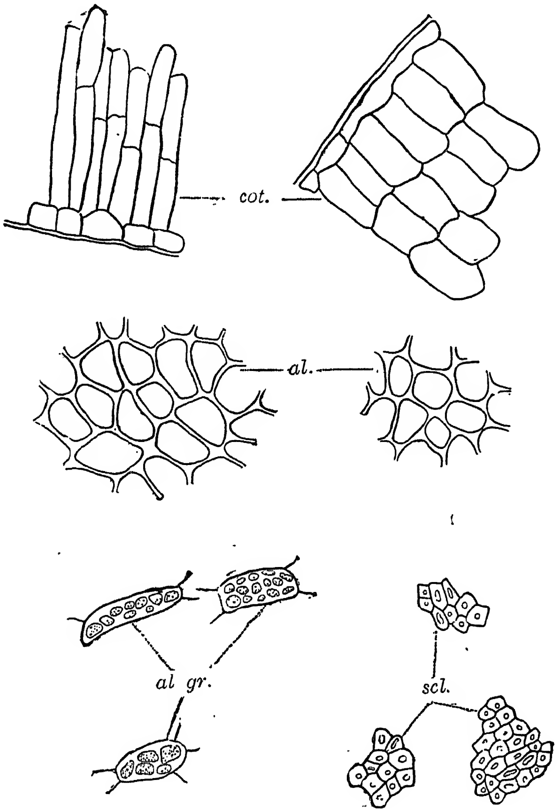


FIG. 108.—White Mustard Seed, fragments from the powder. *cot.*, cotyledons; *al.*, cells of the aleurone layer; *al. gr.*, cells of cotyledon with aleurone grains; *scl.*, palisade cells. ×240.

upwards; the outlines of the thin upper part of the walls may often be detected as a delicate polygonal network. Sometimes layer 2 adheres to the palisade layer, and its cells can then be distinguished on still further raising the focus; in fact, the presence of this layer is often useful as identifying the upper surface of the palisade layer.

(4) Layer No. 4 generally adheres closely to No. 5 (the aleurone layer), and in this peculiarity, as well as in the shape of its cells, it resembles No. 6. The cells are polygonal, very thin-walled, and very inconspicuous (fig. 107, *par* 3).

(5) The aleurone layer is usually easy to find; the cells are rather thick-walled, polygonal, or rounded, and have granular contents (fig. 108, *al.*).

(6) This layer consists of several rows of very thin-walled collapsed cells, and generally adheres firmly to the aleurone layer.

Of these layers the student should particularly study Nos. 1, 2, and 3, as these are very characteristic of white mustard. Most cruciferous seeds resemble white mustard in their general structure, especially in the presence of a palisade layer, and the details of these tissues should, therefore, be carefully noted (compare black mustard).

Examination of the Powdered Seed.—Prepare some powder from white mustard seeds by crushing them in a mortar; free this coarse powder from fixed oil by washing it with ether or any similar solvent; dry it, first by exposure to the air, and finally, for an hour or so, in an air oven, then powder again, and pass the powder through a No. 60 sieve.

Examine the powder first for aleurone grains. Mount in alcohol. The preparation will contain abundance of the minute (9 to 12 μ) isolated aleurone grains.

Mount a little of the powder in solution of picric acid; the aleurone grains stain yellow; many are scattered throughout the preparation, others are still enclosed in the cells. They are usually very distinct if a small drop only of picric acid is used and, after five minutes, a drop of glycerin is added.

Irrigate a stained preparation with very dilute potash; the grains dissolve at once, leaving behind a number of minute globoids.

Mount a preparation in water and irrigate with chloral iodine; observe the presence here and there of a minute starch

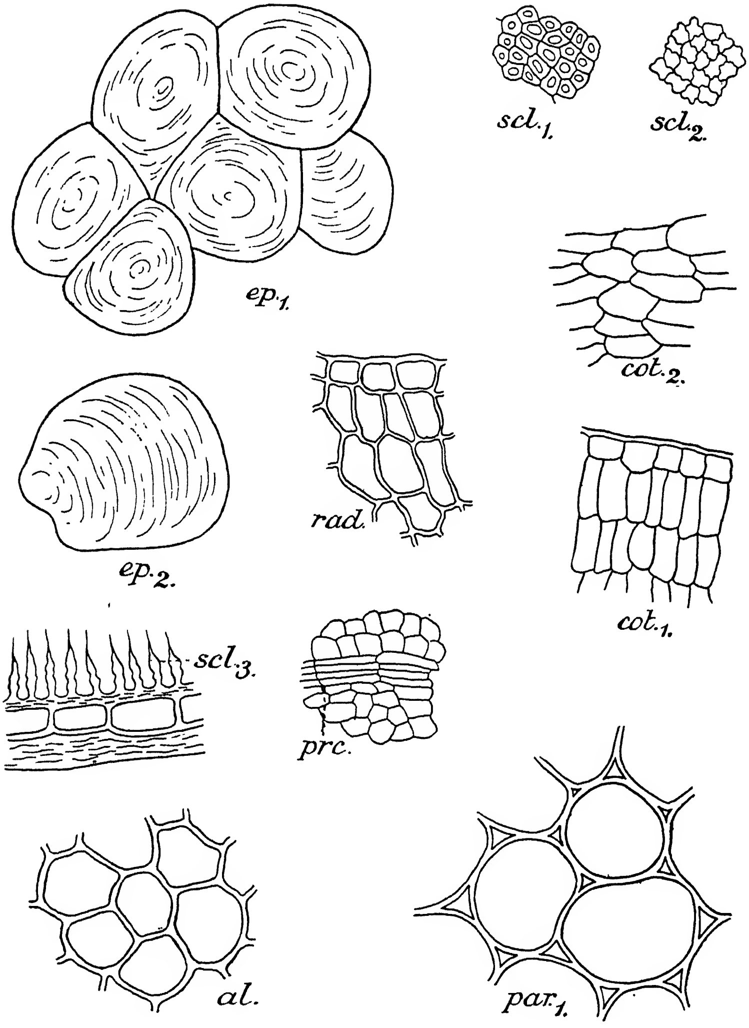


FIG. 109.—Powdered White Mustard Seed. *al.*, aleurone layer, surface view; *cot.*$_1$, parenchymatous cells of cotyledon near the epidermis; *cot.*$_2$, parenchymatous cells of inner portion of cotyledon; *ep.*$_1$, mucilaginous epidermis, surface view; *ep.*$_2$, isolated epidermal cell, side view; *par.*$_1$, parenchymatous cells abutting on the epidermis; *prc.*, procambium with adjacent parenchyma; *rad.*, parenchymatous cells of radicle near the epidermis; *scl.*$_1$, sclerenchymatous cells in surface view at base; *scl.*$_2$, the same at apex; *scl.*$_3$, the same in section with subjacent aleurone layer and collapsed parenchyma. ×240.

grain or a few grains in a group; these are probably from seeds that are not quite ripe.

Moisten a little of the powder with alcohol and irrigate with potash; observe the yellow colour which is produced by fragments of the kernel. Examine this preparation carefully for tissues. The mucilaginous epidermal cells or fragments of them are easily found under a low power, as they stand out against the yellowish liquid, the mucilage remaining colourless and strongly refractive.

The palisade tissue is also very easily identified by its colour and its cells. Layer No. 2 often adheres to this or to the epidermis.

The aleurone layer is colourless, and usually bears attached to it both layers of collapsed parenchyma (No. 4 and No. 6), one on either side.

Much of the *débris* of the cotyledons and radicle can be found in the shape of small masses of delicate parenchymatous cells.

Mount a little of the powder in chloral hydrate; examine in this preparation more particularly the fragments of the cotyledons and radicle; the cell walls of the latter are rather thicker than those of the cotyledons and there are intercellular spaces.

Mount a little powder in ruthenium red; the mucilage stains pink.

Examination of Commercial Mustard.—In preparing the table mustard of commerce the seeds are crushed and the seed coats are almost entirely removed by sifting. Mustard consists, therefore, chiefly of the powdered cotyledons and radicle, with occasional fragments of the seed coats.

In examining it, the following method will be found advantageous:

Defat about 2 grammes of the powder, drain well, and dry by exposure to the air. Reserve a portion of this for examination, as directed for the powdered seed.

Take about 0·5 gramme of the defatted powder and suspend it in a weak (0·5 per cent.) solution of caustic potash, which will dissolve the ground substance of the aleurone grains and clear the tissues. Separation of the cellular *débris* by subsidence is tedious, as the liquid is rather viscid, and recourse must be had to the centrifuge. Wash the deposited cell *débris* once with water, and separate again. The fragments of the seed coats are usually deposited first, as they are comparatively heavy, and they can often be easily distinguished in the point of the tube.

Examine this deposit in dilute glycerin or chloral hydrate. The various tissues are exceedingly clear, and can readily be identified. Any cells or tissues derived from foreign seeds can be much more easily detected in the powder prepared as indicated than in that which has not been so treated.

In exceptional cases it may be desirable to endeavour to remove the delicate parenchymatous tissue and thus concentrate any more resistent cells or tissues, such as sclerenchymatous cells, bast fibres, &c., into a smaller compass. This may be effected by warming the defatted powder with nitric acid and potassium chlorate, stopping the operation as soon as the parenchymatous tissue is judged to be destroyed, and then separating by the centrifuge the tissues that have resisted oxidation. Among these will be the sclerenchymatous layer of the seed coats in a more or less altered condition.

Black Mustard Seed

Examine this seed in the same way as white mustard, and note the following differences:

(*a*) The mucilage in the epidermal cells swells less and is less conspicuous; the striations are not marked.

(*b*) Layer No. 2 (fig. 110, *m*) consists of a single row of cells which do not exhibit the intercellular spaces and collenchymatous thickenings characteristic of those of white mustard.

(*c*) The palisade cells are dark yellowish or reddish-brown in colour; at intervals some are conspicuously elongated; this irregularity makes itself perceptible in indistinct, reticulate outlines on the surface view of the palisade tissue.

(*d*) The cells of layer No. 4 (fig. 110, *p*) contain a dark reddish-brown amorphous substance.

The cells of the cotyledons and embryo and their contents do not differ in appearance from those of white mustard, and the microscope would be unable to distinguish between them if the husk were entirely removed. This, however, is never completely effected, and small fragments of the seed coats are always to be found in the table mustard of commerce.

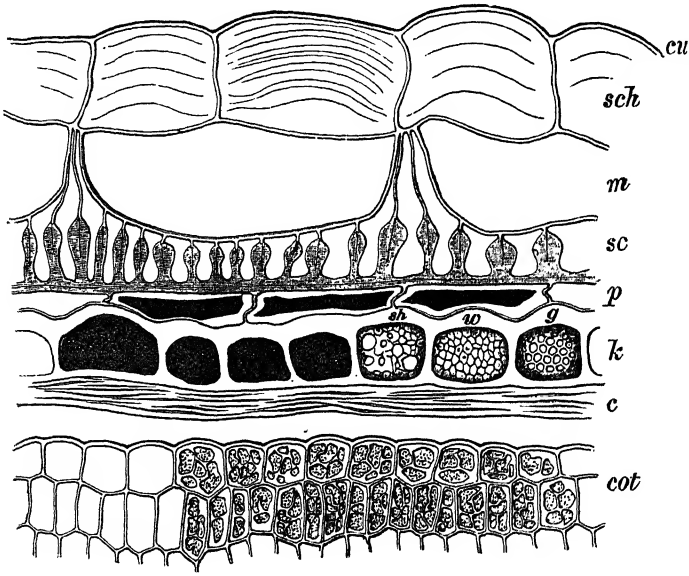


FIG. 110.—Black Mustard Seed, transverse section. *c*, collapsed remains of nucellus; *cot*, cotyledon; *cut*, cuticle; *k*, aleurone layer; *m*, large subepidermal cells; *p*, pigment cells; *sc*, sclerenchymatous (palisade) cells; *sch*, mucilaginous epidermis. (Tschirch.)

Linseed

Source.—The seed of *Linum usitatissimum*, Linn.

Preparation.—The method to be followed in the examination of linseed is similar to that for mustard, but in this case the seed should be fixed in cork for section cutting. The seed coats are very liable to separate from the cotyledons, and often from the endosperm also, but this need not cause any inconvenience.

Examination of Transverse Sections.—A transverse section shows the following tissues:

(1) *Epidermis*, consisting of large cells (width about 30 to 45 μ), which contain mucilage deposited on the outer

and radial walls. In contact with water the mucilage swells considerably, and often exhibits very distinct stratification, which, however, is quite different from that of white mustard; it stains red with ruthenium red. The epidermal cells have very thin radial walls, which suddenly thicken a little near the inner tangential wall (not shown in the illustration); the outer tangential wall is rather thicker, and is provided with a distinct cuticle. (Fig. 112, *Ep.*, in which the mucilage is not shown.)

(2) *Parenchymatous Layer*, consisting usually of two rows of cells (diameter, 20 to 25 μ) which, however, are often partly collapsed; they have rather thick walls, and exhibit intercellular spaces. Towards the sharp edge of the seed there are about five rows of such cells; here they have thinner walls, and are more elongated tangentially. Near one edge the raphe can generally be detected as a small fibrovascular bundle in this tissue. (Fig. 112, *H*, in this case one layer only of cells.)

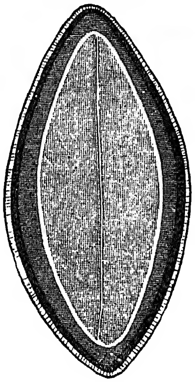


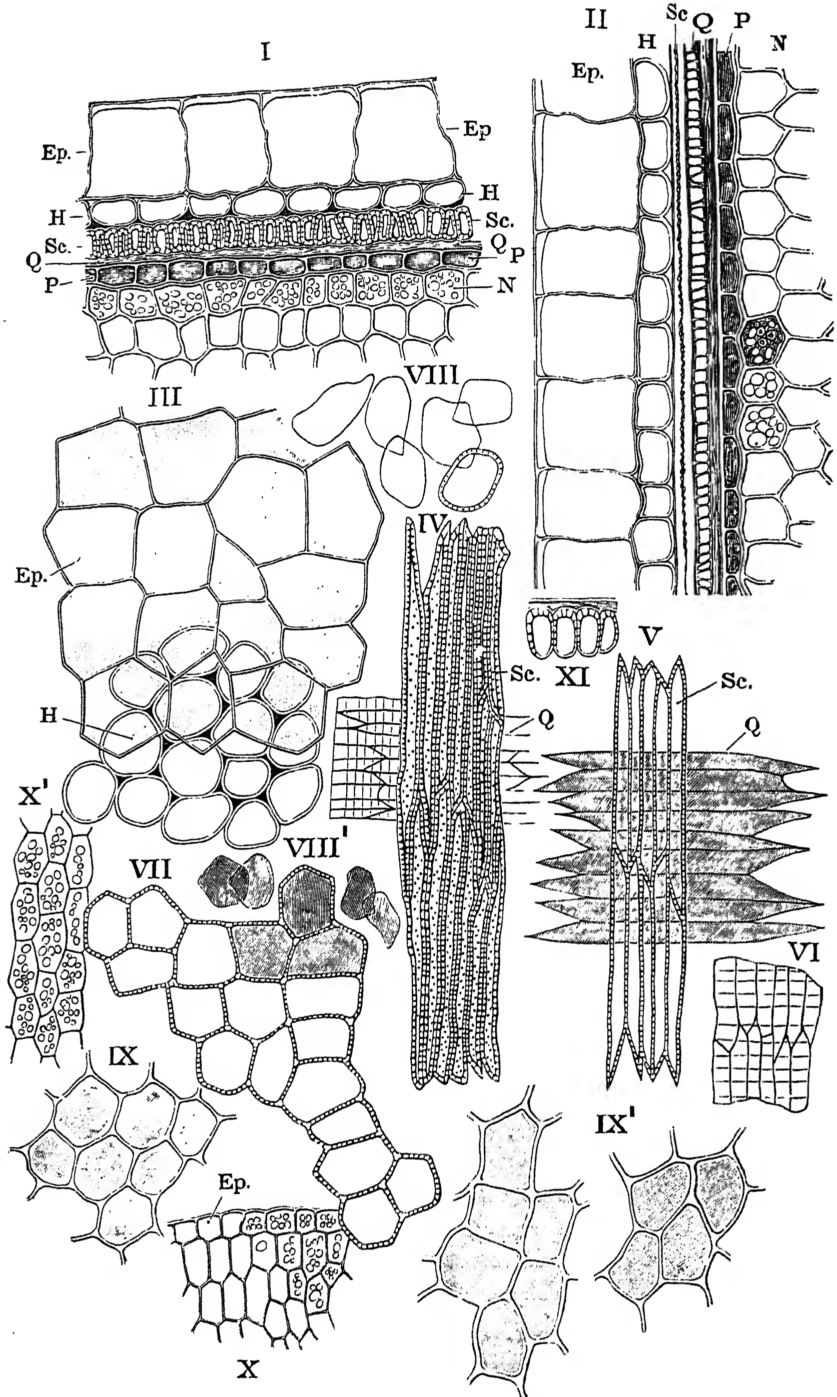
FIG. 111.–Linseed, transverse section, showing the seed coats, endosperm, and cotyledons. Magnified. (Moeller.)

(3) *Sclerenchymatous Layer*, composed of radially elongated cells of yellowish-brown colour. These cells vary in size and appearance in different varieties of linseed and are usually larger and more strongly elongated near the edge of the seed than they are on the flat side. Their walls are thickened, pitted and lignified. (Fig. 112, *Sc.*)

(4) *Hyaline Layer.*—This is narrow and colourless, and exhibits indications only of cell lumina. (Fig. 112, *Q.*)

(5) *Pigment Layer*, which is very conspicuous. It consists of a single row of flattened parenchymatous cells, each of which is completely filled with a homogeneous, dark reddish-brown, amorphous mass. (Fig. 112, *P.*)

(6) *Kernel*, consisting of a narrow endosperm, two cotyledons, and a small radicle. Both cotyledons and endosperm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consist of delicate parenchymatous cells containing aleurone grains and fixed oil.

The cells of the endosperm are approximately isodiametric, and have comparatively thick walls (fig. 112, IX, IX′). The aleurone grains are small and irregular, and do not well exhibit either globoid or crystalloid. In addition to aleurone grains, the cells contain oil, which readily exudes on treatment with chloral hydrate.

The cells of the cotyledons are elongated and have very thin walls (fig. 112, X). They contain very characteristic aleurone grains.

Examination of the Aleurone Grains.—Mount a very thin section in glycerin; the aleurone grains show very distinctly; they are ovoid in form, averaging 10 to 15 μ in length, and contain a large globoid at one end.

Stain a section with iodine or picric acid; the globoid, together with one or two large, obscurely angular crystalloids, can be seen.

Irrigate with dilute solution of potash; the ground substance and crystalloids dissolve at once, leaving only the large rounded globoids. These are conspicuous and characteristic, and serve as a means of identifying fragments of the cotyledon of linseed, even after treatment with ether followed by dilute potash.

Isolation of the Tissues.—The tissues of which the seed coats consist must be isolated and examined as those of mustard seed were. Split one or two seeds open, and remove the kernel. Macerate the seed coats for an hour or more in solution of potash; then vigorously scrape and tease out the tissues.

FIG. 112.—Linseed. I, transverse; and II, longitudinal sections of seed coats and subjacent endosperm cells; *Ep.*, epidermis (mucilage not shown); *H*, subepidermal parenchyma (in this case one layer only); *Sc*, sclerenchymatous cells; *Q*, thin-walled cells crossing these at right angles (compare IV and V); *P*, pigment cells; *N*, cells of endosperm; III, surface view of mucilaginous epidermis, *Ep.*, and subjacent parenchyma *H*; IV and V, surface view of sclerenchymatous cells with thin-walled parenchymatous cells crossing them; VI, the latter cells alone; VII, surface view of pigment layer; VIII, isolated cells of same; VIII′, brown masses of contents of these cells; IX, IX′, endosperm cells in surface view; X, section through the margin of the cotyledon; XI, group of sclerenchymatous cells (figs. III and IV from the powder). (Vogl.)

(1) The epidermal cells are easily identified; they are large, polygonal or oblong, and thin-walled, and contain or are surrounded by transparent mucilage (length, 30 to 75 μ, mostly 40 to 60 μ). (Fig. 112, III, *Ep.*)

(2) The parenchymatous layer (2) usually adheres firmly to the sclerenchymatous layer (3), and is often covered by the epidermis. The cells are rounded and rather thick-walled, with intercellular spaces. Diameter about 30 μ. One row of cells is always distinctly visible, but the second is not easy to detect. Those from the edge of the seed are larger and thinner; such cells cover larger and paler sclerenchymatous cells. (Fig. 112, III, *H.*)

(3) The sclerenchymatous layer is very conspicuous, and is almost always associated with layers (2) and (4). It has a brownish-yellow colour, and consists of very long (200 μ and upwards) and narrow (often 4 to 5 μ) fibrous cells with thickened, lignified, pitted walls. The cavities of the cells are very small, and hence their shape is not very readily seen. Separation by maceration with Schulze's mixture assists in the correct interpretation of them. (Fig. 112, IV, *Sc.*)

(4) This, the hyaline layer, always remains firmly attached to the sclerenchymatous cells, and is best seen at the torn edge of the latter, from which it often projects a little. It consists of long, narrow, colourless, parenchymatous cells with very thin walls, and these cells are themselves crossed at right angles by another similar layer, which, however, can be seen only here and there. (Fig. 112, IV, *Q.*)

Layers (2), (3), and (4) almost always occur together, and are very characteristic of linseed.

(5) The pigment layer is very easily found. The cells are polygonal or oblong (20 to 30 μ), and have straight or slightly curved, rather thick, pitted walls. They are completely filled with a deep brown, homogeneous, amorphous mass, which, however, often falls out intact from torn cells; such cell contents can be found in every preparation. (Fig. 112, VII, VIII, VIII'.)

Examination of the Powder.—Defat some powdered linseed as directed for mustard.

Moisten a little on a slide with alcohol; allow it nearly to dry, and then add a small drop of solution of picric acid; after five minutes add a drop of glycerin, mix well, and examine. The aleurone grains stain yellow, and can be readily identified (the yellow colour is very slowly removed by the glycerin): those of the cotyledons are more conspicuous than those of the endosperm. Irrigate with dilute solution of potash, and observe the globoids that are left.

Mount a little in chloral hydrate (after alcohol). The tissues of the seed coats are usually very distinct. The sclerenchymatous layer is the most conspicuous. With it are commonly associated the parenchymatous layer and epidermis above, and the delicate parenchyma below; the latter tissue is best seen at the edges of the fragment, where it projects beyond the sclerenchyma; the other layers can usually be seen by suitable focussing.

Very conspicuous also are the quadratic cells of the pigment layer, with their pitted walls; the brown, amorphous masses of contents are also easily found.

The parenchymatous tissue of the cotyledons can be identified particularly easily by the numerous large rounded globoids left after solution of the ground substance and crystalloids; these are very conspicuous. Similar globoids are to be found scattered over the field.

The cells of the endosperm are less elongated; they have rather thicker walls, and less conspicuous globoids.

Mount a little in ruthenium red; the masses of mucilage stain pink.

Nux Vomica Seeds

Source.—The seeds of *Strychnos Nux-vomica*, Linn.

Preparation and Examination of Sections.—Split some seeds in half, and soak them, together with a few whole seeds, in water for twelve hours or more. Expose also some split seeds to a moist atmosphere for twenty-four hours. Take a suitably softened half-seed, and cut it into two semicircular pieces by an incision passing through the centre. As the hairs assume a radial arrangement, this incision will be parallel to them, and this can easily be determined by examination with a lens. Now cut a narrow strip by two incisions at right angles to the radial cut; sections from the flat end of the strip will then be parallel to the direction which the hairs assume. Embed this

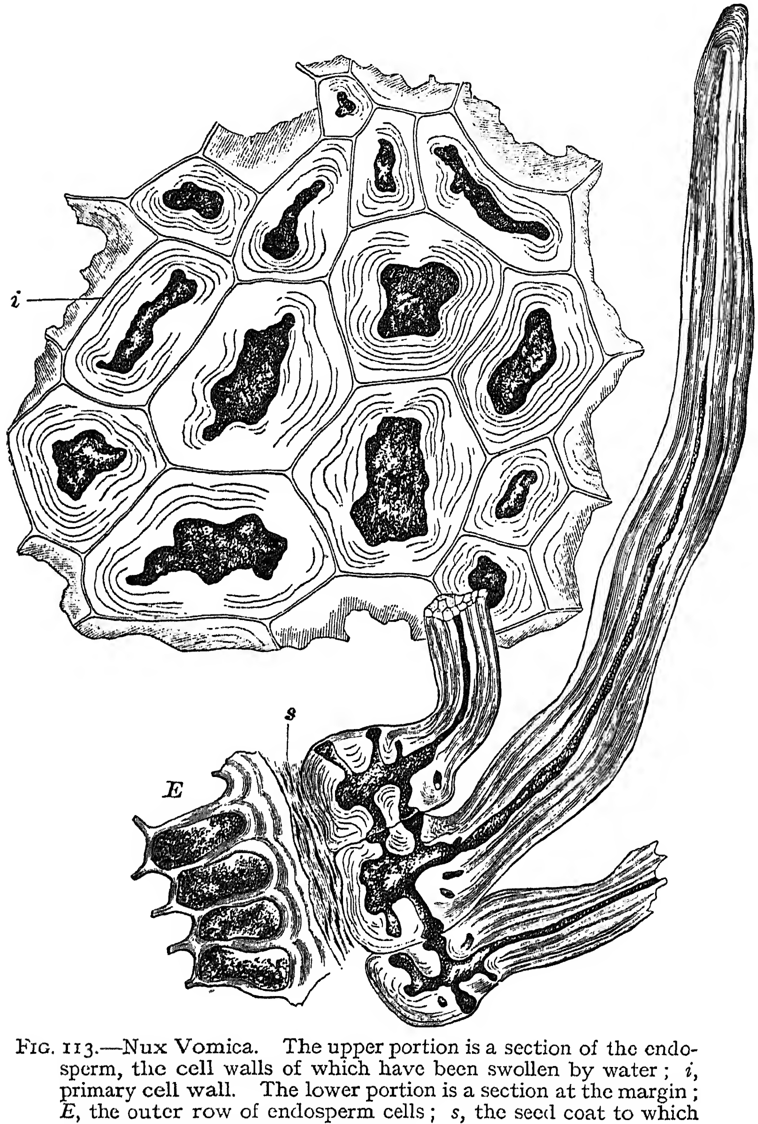

FIG. 113.—Nux Vomica. The upper portion is a section of the endosperm, the cell walls of which have been swollen by water; *i*, primary cell wall. The lower portion is a section at the margin; *E*, the outer row of endosperm cells; *s*, the seed coat to which the hairs are attached. (Moeller.)

strip in pith, and cut sections, taking care that the razor follows the hairs from base to apex, and transfer them to alcohol.

Mount a section in water. The outermost layer consists of the epidermal cells, which have developed into remarkable hairs ; these are bent over near the base, and closely appressed to the seed, thus giving it its silky appearance. These hairs cannot, however, be satisfactorily studied from a section, as many of them will have been cut, and in any case the basal part is indistinct. They will be more closely examined later.

Following the epidermis is a narrow brown layer, in which indications of collapsed cells can be discerned. This layer can be isolated by warming with potash, and is then seen to consist of ill-defined, delicate, thin-walled, polygonal cells.

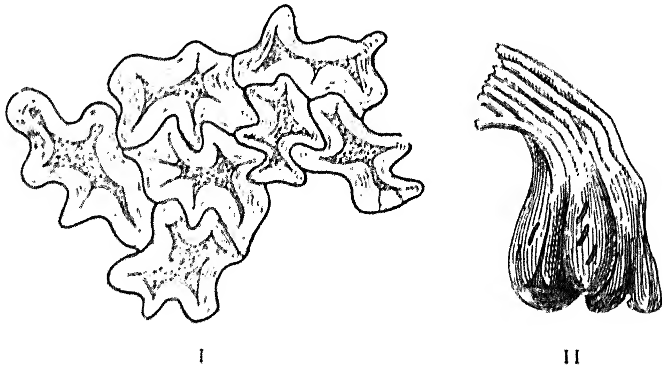

Fig. 114. Nux Vomica. I, hairs viewed from below, showing the transverse sections of bases; II, base of hair viewed from the side. × 240.

These two layers comprise the whole of the integuments of the seed, and are directly followed by the endosperm, which is next to be examined.

In a section mounted in water observe that the endosperm cells are large, and have very thick, strongly refractive walls. Those at the periphery are smaller than the inner ones, and more elongated, forming a kind of palisade. The cells have granular contents.

Cut some sections from a seed that has not been soaked in water ; flatten them out as well as possible, and mount in alcohol. In the cells irregular, angular, granular masses can be seen, often two, three, or four together in one cell. These are the aleurone grains. Irrigate with iodine water ; the cell walls swell ; the aleurone grains acquire a yellow colour and become more distinct. Irrigate with dilute potash; they dissolve, leaving behind a few minute globoids.

Mount another section in water and irrigate with solution of potash; the cell contents assume a yellow colour, due probably to the caffeotannic acid present.

Mount a section in alcohol. Focus under the low power. Irrigate it with water; the cell walls swell distinctly. Warm the section till the water just begins to boil; the cell walls swell still more strongly, often completely obliterating the cavity and squeezing out the contents.

Stain another section with solution of iodopotassium iodide, wash, and irrigate with concentrated sulphuric acid; the cell walls assume a blue colour. These tests indicate that the cell walls are composed of a mucilaginous modification of cellulose.

Next study more carefully the hairs.

Shave off from the surface of a seed the epidermis (with part of the subjacent endosperm). Digest for a few minutes with potassium chlorate and nitric acid, wash, and transfer to spirit.

Examine a fragment in glycerin, placing it with the hairy side downwards. In favourable pieces the outlines of the epidermal cells can be seen; they are usually very irregular, wavy, or jagged, and often bear knob-like projections, which can be seen by focussing upwards.

Tease out another part into its component hairs. Observe their remarkable shape. The basal part is very irregular (compare with previous preparation), often bearing projections below and oblique pits on the sides. The upper part is drawn out into a very long hair (1,500 μ), the wall of which bears on its inner surface a number of nearly parallel thickened bands which anastomose but little, and finally meet in the rounded apex of the hairs; these bands are lignified. In many hairs the oxidising mixture has destroyed the intervening strands of cellulose, and the hairs fray out into their component bands towards the apex; some, however, will be intact.

Mount in water a section that has not been immersed in alcohol; remove the water, and irrigate with sulphovanadic acid; the contents of the cells rapidly assume a violet coloration (reaction for strychnine).

Examination of the Powder.—Mount a little in water. Observe the fragments of endosperm and of endosperm cells, which are conspicuous by reason of their very thick walls. Irrigate with water; the cell walls swell and the structure becomes clearer. Irrigate with solution of potash; the cell walls swell still further and a bright yellow colour is developed.

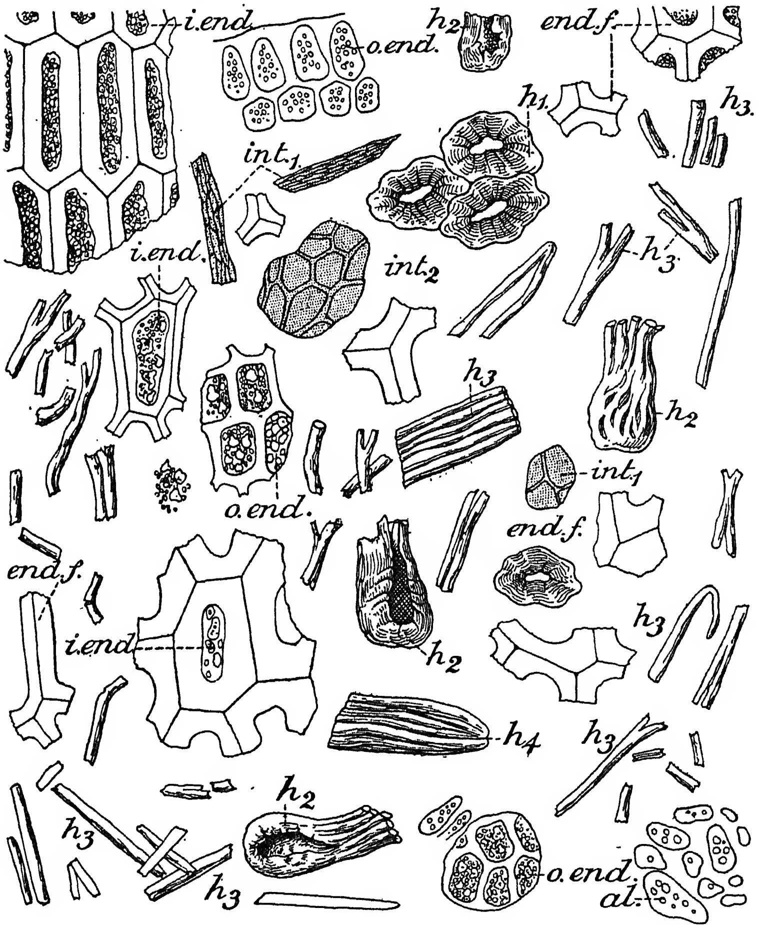

FIG. 115.—Powdered Nux Vomica. *al.*, aleurone grains; *end.f.*, fragments of endosperm; *i.end.*, inner portion of endosperm; *o.end.*, outer portion of endosperm; h_1, bases of hairs in surface view from below; h_2, the same in section; h_3, fragments of hairs; h_4, apex of hair; *int.*$_1$, seed coat, in section; *int.*$_2$, the same in surface view. (Partly after Greenish and Collin.) × 200.

Mount a little in dilute glycerin, allowing it to stand, if possible, twelve to twenty-four hours. Examine under the low power. Note the colour; most of the particles are colourless, but some are pale or even dark brown. Conspicuous in the powder are a number of transparent, colourless, rod-like bodies about 5 to 15 μ wide and up to 100 μ long; they are the thickened portions of the hairs which have been broken up by the grinding. In addition to these rods, numerous colourless fragments of the thick walls of the endosperm cells are to be seen as well as larger fragments of endosperm, the cells of which have granular contents. Further examination will reveal the presence of brownish irregular bodies; these are probably the bases of the hairs and will show their structure better in the following preparation.

Mount a little of the powder in chloral hydrate; allow the preparation to stand a few minutes, or warm it gently. The walls of the endosperm cells, especially the thicker, swell considerably and become almost invisible; the hair bases, on the other hand, become more distinct and their structure clearer; most of them exhibit their profile, but fragments may be found exhibiting the outlines of the cells.

Stain a little of the powder with phloroglucin and hydrochloric acid; the hair bases and the fragments of the hairs stain more or less deep pink, and become more conspicuous. They are most clearly seen after treatment of the powder with nitric acid and potassium chlorate, by which the walls and contents of the endosperm cells are destroyed.

The aleurone grains are not readily distinguished. Moisten a little of the powder with alcohol; add a drop of solution of picric acid; allow it to remain a few minutes, add a small drop of glycerin, and examine. The aleurone grains are stained yellow; they are rounded, oval or irregular in shape, mostly 10 to 30 μ in diameter, and contain one or more globoids.

Areca Nut

Source.—The seeds of *Areca Catechu*, Linn.

Preparation and Examination of Sections.—Select one or two areca nuts that have part of the yellowish inner layers of the pericarp adhering to them, and soak them for forty-eight hours in water or till they are sufficiently soft to cut. Cut transverse sections from the outer part of a seed, taking care to include the remains of the pericarp. Transfer the sections to alcohol. Mount one in chloral hydrate.

The outermost layer consists of elongated [illegible] slightly thickened, pitted, and lignified walls. [illegible] in size, but often measure about 120 to 150 μ in length and 15 to 20 μ in breadth. They exhibit intercellular spaces and easily separate from one another; hence they may be found loose, or nearly so, and hence also the surface of the remains of the pericarp is scurfy.

This layer is usually from two to six cells wide, and is bounded on the inner side by a single row of parenchymatous cells which present a nearly square or slightly radially elongated section. These cells vary from 10 to 15 μ in width, and have pitted, strongly lignified walls. Surface sections show them to be polygonal and approximately isodiametric. This layer is the inner epidermis of the pericarp, and hence the tissues hitherto observed do not constitute part of the seed proper.

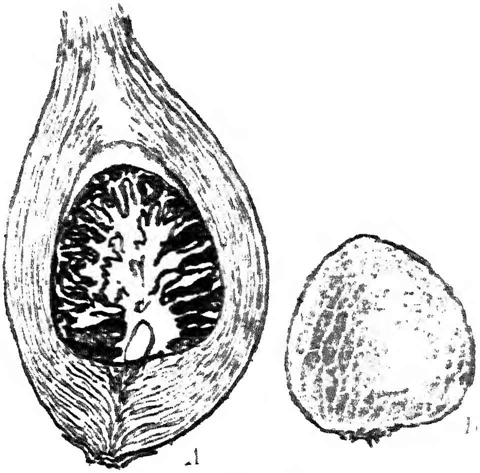

FIG. 110.—Areca Nut. *A*, vertical section of the fruit and seed showing the fibrous pericarp of the former and ruminate endosperm of the latter; *B*, seed. Natural size. (Bentley and Trimen.)

Following close upon the inner epidermis of the pericarp are the seed coats. These average about 300 μ in thickness, and are rather sharply differentiated into two layers, an outer thick-walled and an inner thin-walled layer. The cells of the outer layer present an oval, rounded, or elongated section, due to the irregular direction of the cells ; between them there are often intercellular spaces, or even cavities of considerable size, caused by the destruction of delicate parenchymatous tissue which originally filled them. Those cells that abut on the pericarp are often compressed or collapsed, and hence not well defined ; here and there elongated narrow cells can be found ; the epidermis is seldom distinguishable. The cells next to these exhibit rounded, oval, or even elongated sections ; they measure from 10 to 15 μ in diameter, and have comparatively thick walls.

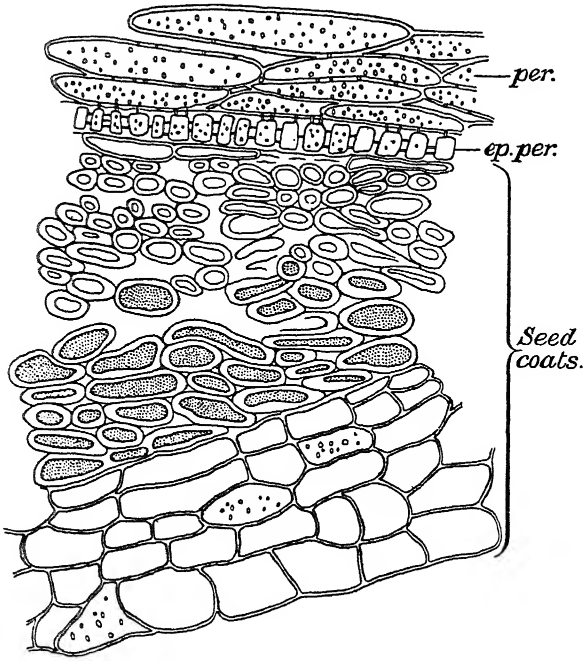

FIG. 117.—Areca Nut, transverse section of seed coats. *per.*, inner layers of cells of pericarp ; *ep. per.*, inner epidermis of pericarp. × 240.

Towards the centre of the seed coat the cells are larger, measuring 20 to 60 μ in length, the cavities are larger, and the walls are not so thick in proportion to the size of the cells as are the walls of the smaller cells. All the cells of this outer, thick-walled layer, especially those of the inner part, have dark reddish-brown cell contents, and often reddish-brown walls ; this imparts to the seed coat its characteristic colour.

In order to obtain an insight into the [illegible] and shape of these cells, it is necessary to separate them. This [illegible] be done by gently scraping off the seed coat, moistening the powder so produced with alcohol, and finally examining in glycerin. The cells exhibit the greatest variety of outline: some are very long and narrow, many are elongated and have moderately thick, pitted walls, others are nearly isodiametric. They are not regularly arranged, but often interlace, thus producing the varying sections shown in the illustration. The walls are often irregularly thickened but not striated.

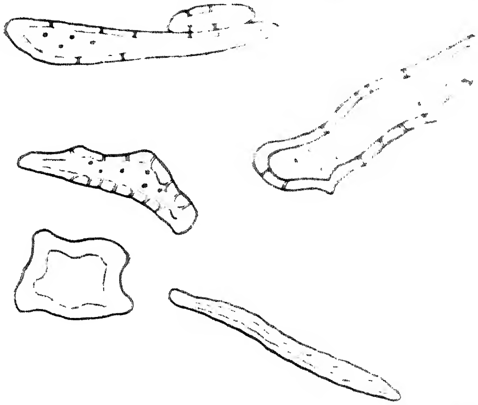

FIG. 118.—Areca Nut. Cells isolated from outer part of seed coat. × 200.

The inner, thin-walled part of the seed coat consists of larger cells with thinner, pitted walls, and contents that are less deeply coloured. Tissue of this nature, but often consisting of smaller and more or less collapsed cells, constitutes the main portion of the ruminations which penetrate the endosperm. The cells, especially those abutting on the endosperm, contain a homogeneous reddish-brown substance that reacts for tannin. At the point where each of these processes diverges from the seed coat there is usually a rather large fibrovascular bundle.

The cells of the endosperm are polygonal and nearly isodiametric, varying very much in size (30 to 120 μ, many about 60 μ). They have very thick cellulose walls (10 μ), which swell in contact with water; the pits are large and rounded (6 to 12 μ), and often resemble bordered pits in section. They contain a granular proteid matter, which stains yellow with iodine and dissolves in very dilute potash. Many crystals of fat can be distinguished; these melt when warmed, and then stain with appropriate reagents.

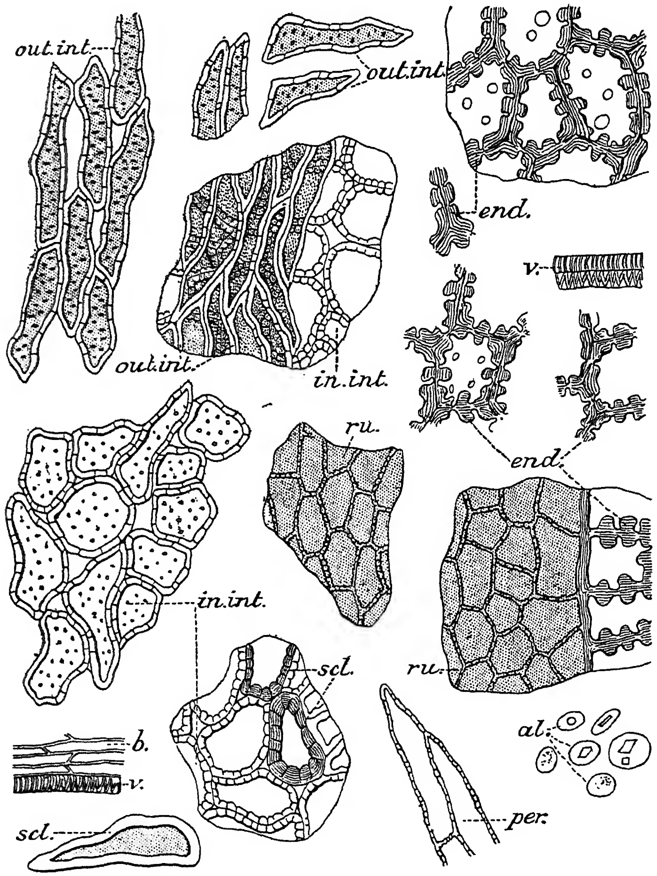

FIG. 119.—Powdered Areca Nut. *al.*, aleurone grains; *b.*, bast; *end.*, endosperm; *in. int.*, cells from inner layers of seed coat; *out. int.*, cells from outer layers of seed coat; *per.*, cells of pericarp, adhering to seed; *ru.*, cells of ruminations; *scl.*, sclerenchymatous cells; *v.*, vessel. (Partly after Greenish and Collin.) ×150.

Examination of the Powder.—Mount a little in dilute glycerin; allow it to stand twelve to twenty-four hours and examine with the low power. Observe the colour. Many of the smaller fragments are colourless or nearly so (endosperm cells), but many are pale to reddish or brown (ruminations and seed coats); larger fragments often exhibit colourless walls with greyish or brownish cell contents. Examine further with the high power; the endosperm cell walls are colourless and exhibit remarkable knob-like projections or large, characteristic, rounded pits; even the smallest fragments may be identified. Elongated cells (from the seed coat), usually with reddish contents, may also be found. The larger fragments exhibit their structure better in chloral hydrate.

Mount a little in chloral hydrate; allow it to stand without warming. The colourless or nearly colourless fragments of the endosperm are now clear and can be readily identified. The brownish or reddish portions of the seed coat and ruminations are gradually decolourised and become clearer. Those from the ruminations are the most numerous; their cells have only moderately thick walls, with numerous rounded or pointed pits; they are irregular in shape, being sometimes nearly isodiametric, sometimes elongated. The cells from the outer portion of the seed coat are mostly elongated and pitted, but vary much in both respects.

The aleurone grains can best be seen by staining as follows: Defat a little of the powder by shaking in a tube with a mixture of ether (2 volumes) and alcohol (1 volume); let it deposit; pour off the clear liquid and transfer a little of the moist powder to a slide. Add a drop of aqueous solution of eosin, mix, let the mixture nearly dry, add a drop of glycerin and examine. The aleurone grains (and also the remains of the plasma) stain bright pink; they are oval or rounded and average about 20 μ in diameter; each contains one or more crystalloids; many are free in the powder, but some are still contained in the endosperm cells.

Cocoa

Source.—The seeds of *Theobroma Cacao*, Linn.

Examination of the Kernel

Endosperm.—Remove the shells from a few cocoa beans, and soak the kernels in water; reserve the shells.

Examine the surface of the cotyledons; note the soft, pale membrane that adheres to it, and also penetrates the folds of the cotyledons. This is the remains of the endosperm.[1] Remove a portion of that which is on the outside, mount in water, and examine. There is only one layer of cells, those of the epidermis, to be distinctly seen; these are polygonal, about 20 to 30 μ in diameter, and contain a greyish, granular substance, occasional small crystals of calcium oxalate, and sphærocrystalline masses of fat. Adhering to the inner surface of this membrane are remarkable scattered hairs; they are club-shaped, and consist of a single or, in the upper part, double row of short rounded cells containing brown granules. These hairs may be 100 μ long and 20 to 30 μ wide; they are developed from the epidermal cells of the cotyledon, but break off and adhere to the remains of the endosperm. The rest of the membrane consists of a confused mass of delicate collapsed parenchyma. The fatty nature of the sphærites above mentioned may be ascertained by their melting into globules when warmed, and by their acquiring a brown colour when irrigated with osmic acid.

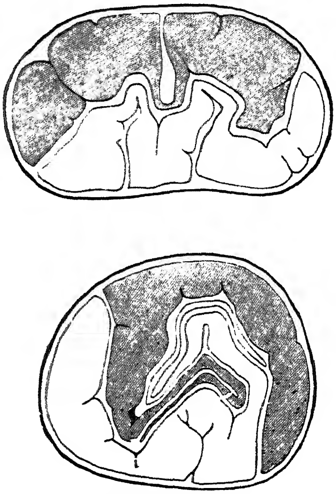

FIG. 120.—Cocoa Seeds. Sections showing the folding of the cotyledons. (Tschirch.)

That part of the endosperm which penetrates between the folds of the cotyledons is similar to that which covers them externally, but is devoid of the epidermis.

Cotyledons.—Remove the endosperm from part of the cotyledons, and from the surface of the latter cut very thin surface sections; mount them in glycerin, keeping the epidermis uppermost. Examine the epidermis. It consists of polygonal, isodiametric, or oblong cells measuring about 15 to 30 μ, with thin walls. Conspicuous among the contents are little rounded granules of deep reddish-brown colour; to these the dark colour of the outer layer is due.

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[1] Tschirch, *Anatomischer Atlas*, p. 22.

Examine a transverse section of the outer part of the cotyledon; the epidermal cells are flattened; they are characterised by the reddish-brown granules they contain. Below the epidermis the cells are polygonal, about 30 μ in diameter, and appear rather thick-walled; this, however, is partly due to an adhering layer of protoplasm, which can be stained yellow by iodine. Chloral hydrate clears the section well, so also does chloral iodine; in the latter reagent the cell walls appear thin, and in each cell numerous small starch grains stain blue.

Mount a section in water, tearing it so as to liberate the starch grains, and examine them; they are very small and mostly rounded; many are simple, but some are compound.

Examine more closely a section mounted in water. In addition to starch grains, most of the cells contain large, transparent, colourless masses which show a more or less distinctly radiate structure. Irrigate a section with solution of osmic acid; they slowly acquire a brown or nearly black colour. Warm a section in water; they melt to globules. These tests indicate them to be crystalline masses of fat.

Boil a section gently in chloral hydrate for a few moments; cool, and, if necessary, add more chloral hydrate to replace that which has been lost. The starch, fat, and remains of protoplasm dissolve more or less completely. Here and there small crystals of calcium oxalate can be detected.

Defat some sections by maceration in ether-alcohol, wash with alcohol. Mount one in water, and irrigate with iodine water. The distribution of the starch can now be well seen. Sometimes the aleurone grains also will be visible after staining with iodine, but they are not easily seen. They are usually small, and each contains a comparatively large globoid.

Examine again the section mounted in water; observe here and there cells or groups of cells containing a reddish amorphous substance. This is cocoa-red. It dissolves in sulphuric acid and in chloral hydrate with red coloration.

The presence of the following cell contents has therefore been determined in the tissue of cotyledon:

(*a*) Fat, in crystalline masses, in most of the cells.
(*b*) Starch grains, very small, simple or compound, in most of the cells.
(*c*) Calcium oxalate crystals, here and there.

(*d*) Aleurone grains, small, with large globoids, not easily detected.

(*e*) Cocoa-red, restricted to isolated cells or groups of a few cells.

Examination of the Shells

Sections.—Expose some cocoa shells to a moderately moist atmosphere until they lose their rigidity, but not longer, as they are apt to become inconveniently moist. Cut transverse sections between pith, transfer to alcohol, mount in chloral hydrate.

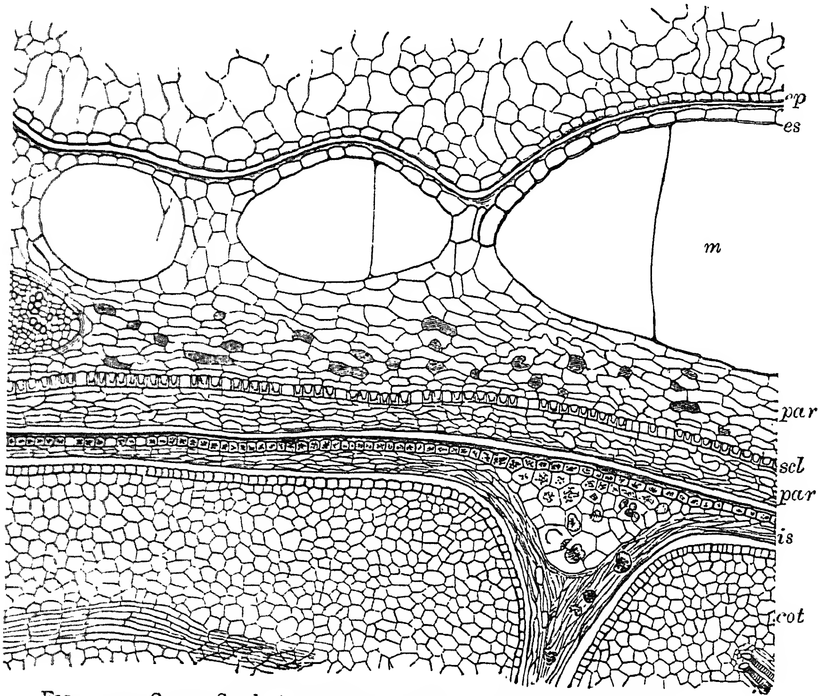

FIG. 121.—Cocoa Seed, transverse section through the periphery of the seed, the seed coats, and the adherent pulp. *cot*, cotyledon; *ep*, inner epidermis of pulp of pericarp; *es*, outer epidermis of seed coat; *is*, remains of endosperm which penetrates between the cotyledons; *m*, mucilage; *par*, parenchymatous tissue of seed coats; *p*, pulp of pericarp; *s*, inner seed coat; *scl*, inner epidermis of outer seed coat. ×45. (Tschirch.)

The sections are very narrow. On the outside there is a confused tissue, in which tubular hyphæ of fungi, numerous minute, rounded isolated cells (yeast cells) and occasional crystals can be detected. This is followed by a layer of collapsed cells several rows thick, which show but little structure in sections, but in favourable surface preparations can be seen to be long, tubular and very thin-walled; on the inner side this tissue is bounded by a row of cells which are more or less conspicuous by reason of the brown colour of their walls. These cells are very small (about 4 to 6 μ in tangential diameter), rectangular, and somewhat flattened in section; they constitute the inner epidermis of the pericarp of the cocoa fruit (fig. 121, *ep*). The latter contains when ripe a scanty pulp, part of which adheres to the seed throughout the processes of fermentation and drying, during which the fungi and fermentative cells, which were observed on the exterior, are developed. All the tissue up to and including the epidermis of the pericarp, although always found on the outer surface of the shell, forms no part of the seed coats. The latter consist of the following layers:

(*a*) The epidermis of the outer seed coat (fig. 121, *es*); the cells are pale in colour and much collapsed; hence they are often only indistinctly visible in section, but they can be made more distinct by bleaching the section with solution of chlorinated soda. They are much larger than the epidermis of the pericarp, measuring about 30 μ in tangential diameter.

(*b*) Single row of very large mucilage cells, very conspicuous by reason of the large quantity of transparent yellowish mucilage secreted by them (fig. 121, *m*). The cell walls are very thin, and most of them have broken down so as to form large oval cavities separated from one another by transverse belts of three or four rows of parenchymatous cells. Ruthenium red colours the mucilage brilliant pink.

(*c*) A mass 150 to 200 μ thick of collapsed parenchyma, through which large fibrovascular bundles run; these bundles are rich in spiral vessels varying in size from 5 to 15 μ.

(*d*) A single row of small cells about 10 to 15 μ in tangential diameter, exhibiting a flattened section and a horseshoe thickening on the inner and radial walls. This layer is the inner epidermis of the outer seed coat (fig. 121, *scl*).

(*c*) The tissue following this is the inner seed coat. It consists of collapsed parenchyma in which there is no perceptible differentiation.

Surface Preparation.—The next step is the study of these cells and tissues in surface preparations.

From some of the same material cut successive surface sections as thin as possible. Place them in succession upon a slide, with the outer side uppermost, and keep them moistened with alcohol for a few minutes; finally, mount in chloral hydrate.

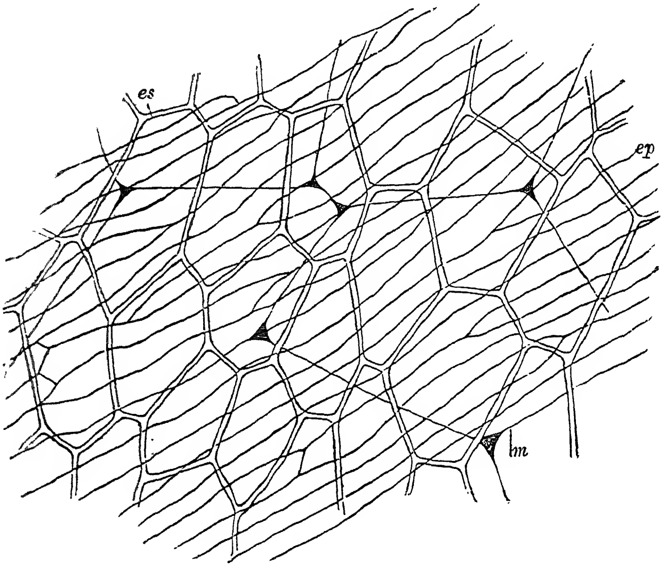

FIG. 122.—Surface view of the epidermis of pericarp and of the seed coats, together with the subjacent mucilage cells. *ep*, epidermis of pericarp; *es*, epidermis of seed coat; *m*, mucilage. ×200. (Tschirch.)

The outer (upper) part consists of an indistinct tissue in which yeast cells, small crystals, and the hyphæ of fungi can be detected. Next to this is a layer of long narrow cells with pointed ends, the inner epidermis of pericarp (fig. 122, *ep*); this tissue is usually easily found. Abutting upon this is the outer epidermis of the outer seed coat (fig. 122, *es*); in surface view the cells are large, polygonal, elongated, and thin-walled; they cross the layer above them, sometimes at right angles but more often diagonally. The walls of the mucilage cells which follow next are not easily seen, but there are [illegible] translucent masses of mucilage.

The nature of the parenchyma that constitutes the bulk of the seed coats is not very readily made out from sections, but the inner epidermis of the outer seed coat is generally distinct. In surface sections the cells appear small, elongated, polygonal (about 10 μ by 20 μ), and thick-walled.

Separation by Digestion.—Next proceed to disintegrate the tissues of the shells. Digest a few fragments with a 1 per cent solution of caustic potash in the water-bath for ten or fifteen minutes (longer if necessary). Tease out a small fragment on a slide with the dissecting needles, and separate the different layers by pressing the fragments firmly with the flat handle of a scalpel. Examine in water or dilute glycerin.

The most conspicuous feature is the presence of numbers of large, rounded, parenchymatous cells, varying mostly from 50 to 100 μ in diameter. The walls are usually of a pale reddish-brown colour, and exhibit short protuberances, by which they were attached to neighbouring cells; these protuberances, seen from above, appear as distinct circles. These cells are derived from the parenchymatous tissue of the shell.

Portions of the outer epidermis of the outer seed coat are also easily found. The cells adhere to form large plates; they are large, polygonal, elongated, and have dark-brown straight or slightly curved walls. This tissue is often crossed by the long narrow cells of the inner epidermis of the pericarp, the two tissues generally remaining firmly adherent.

The sclerenchymatous cells of the inner epidermis of the seed coat may also be found, but they are usually much less conspicuous than the parenchymatous cells above described. After the treatment with potash the lumen appears traversed by one or two narrow bars, probably folds of the radial walls.

Lastly numerous spiral vessels, from 10 to 20 μ wide, can be found, and also the *débris* of tubular cells from the pericarp.

Examination of Powdered Shells

Prepare some powder from the shells, and examine it as follows:

(1) In water; observe the numerous yellowish or brown masses, in which little structure is discernible, and

masses of transparent mucilage, usually tinged with brown. Mounted in solution of ruthenium red, even the smallest particles of mucilage stain brilliant pink or bright red.

(2) After warming in chloral hydrate, the structure becomes clearer; portions of the sclerenchymatous inner epidermis, of the outer epidermis of the seed coat, and of the inner epidermis of the pericarp can be detected. The parenchyma can be identified as well as *débris* from the tissue of the pericarp. There are very numerous fragments of spiral vessels.

(3) Warm a little with solution of potash and endeavour to disintegrate the tissues by pressing upon and moving the coverslip. This preparation usually allows of easy separation of the various layers, and their identification.

Diagnostic Characters.—The following are the chief diagnostic characters of the powdered shells:

(*a*) The two epidermises; long narrow cells crossing larger polygonal ones, often diagonally.

(*b*) The small, polygonal, thick-walled cells of the sclerenchymatous layer.

(*c*) The large, rounded parenchymatous cells with arm-like projections.

(*d*) The mucilage.

Examination of Powdered Cocoa

Having thus thoroughly examined the tissues and cell contents of the kernel and the shell, proceed to examine the powder as follows:

(1) Moisten a little thoroughly with water by continued stirring; mount, and examine in water. There are numerous isolated starch grains, the characters of which can be best examined in water. There are also numerous small and larger masses of tissue in which occasional dark-brown cells can be seen, but little that is definite. These dark-brown cells turn crimson-red with concentrated sulphuric acid.

(2) Heat the preparation to the boiling-point; numberless globules of oil separate. The starch, however, gelatinises with difficulty, and, even after boiling, only the centre of the grains is

translucent. The tissues are clearer, and fragments of the red-brown epidermis, of the cotyledons, and also of the endosperm can be detected. The tissues are, however, not yet clear enough for satisfactory examination.

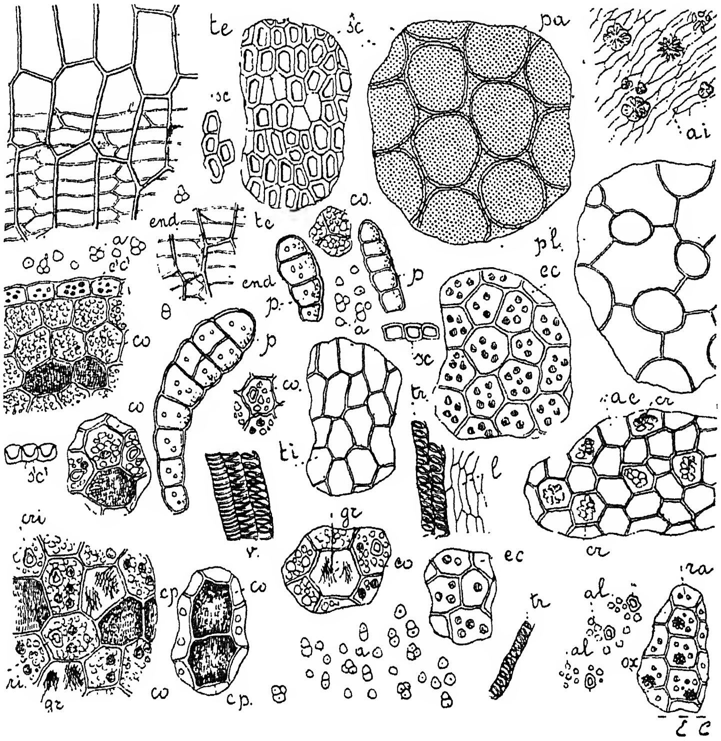

FIG. 123.—Powdered Cocoa Seeds (including seed coats). *a*, starch grains; *ae*, outer layer of endosperm; *ai*, inner layer of endosperm; *al*, aleurone grains; *co*, cotyledon; *cp*, pigment cells containing cocoa-red; *cr*, crystals of fat; *ec*, epidermis of cotyledon, surface view; *e'c'*, epidermis of cotyledon, profile; *end*, inner epidermis of pericarp; *gr*, crystals of fat; *l*, bast from fibrovascular bundles; *ox*, calcium oxalate crystals; *p*, pluricellular hairs; *pa*, *pl*, parenchyma of seed coat; *ra*, cells of radicle; *sc*, sclerenchymatous layer of seed coat, surface view; *s'c'*, sclerenchymatous layer of seed coat, in profile; *te*, outer epidermis of seed coat to which the inner epidermis of the pericarp (*end*) is adhering; *ti*, inner epidermis of seed coat; *tr*, *v*, vessels, &c. × 240. (Greenish and Collin.)

(3) Defat some powder by shaking it with ether-alcohol for a few minutes; wash with alcohol, mount in water. The tissues are clearer than they were in water; add chloral iodine, they

become still clearer and the starch colours blue. Boil gently; they now become quite clear. The delicate colourless cell walls of the cotyledons, the pale to dark-brown epidermis, and the small crystals of calcium oxalate are easily observed. There are occasional, but not numerous fragments of fibrovascular bundles, but only few of the characteristic hairs are to be found.

Diagnostic Characters.—The chief diagnostic characters of the powdered kernels are:

(*a*) The thin-walled parenchyma of the cotyledons.
(*b*) The minute starch grains, either simple and rounded, or compound with two or three component grains; they are difficult to gelatinise.
(*c*) The polygonal epidermal cells of the cotyledons, with dark reddish-brown, granular contents.
(*d*) The characteristic hairs, which, however, are sometimes rare.
(*e*) The abundance of fat.
(*f*) The cells containing red-brown cocoa-red.

Coffee Beans

Source.—The seed of *Coffea arabica*, Linn.

The commercial coffee bean consists chiefly of the endosperm of the seed, the seed coats having been removed by the preparation the beans undergo. Small fragments, however, of the seed coats may be found in the groove running along the flat side of the bean.

Examination of the Seed Coats.—Soften some raw coffee beans by soaking in water for several hours, or in a mixture of equal parts of alcohol and glycerin for twenty-four to forty-eight hours.

Cut one longitudinally through the furrow on the flat side; from the sides of the groove thus opened, strip a small piece of the silvery seed coat. Mount in dilute glycerin or in chloral hydrate.

It is composed of several layers of delicate collapsed parenchymatous cells, but the structure is not very easily seen, a cell wall being visible here and there. In this tissue there are numerous sclerenchymatous cells. Examine these carefully, as they are very characteristic of coffee and always found in it.

They are mostly about eight times as long as they are broad, and sometimes they are much broader in proportion than this.

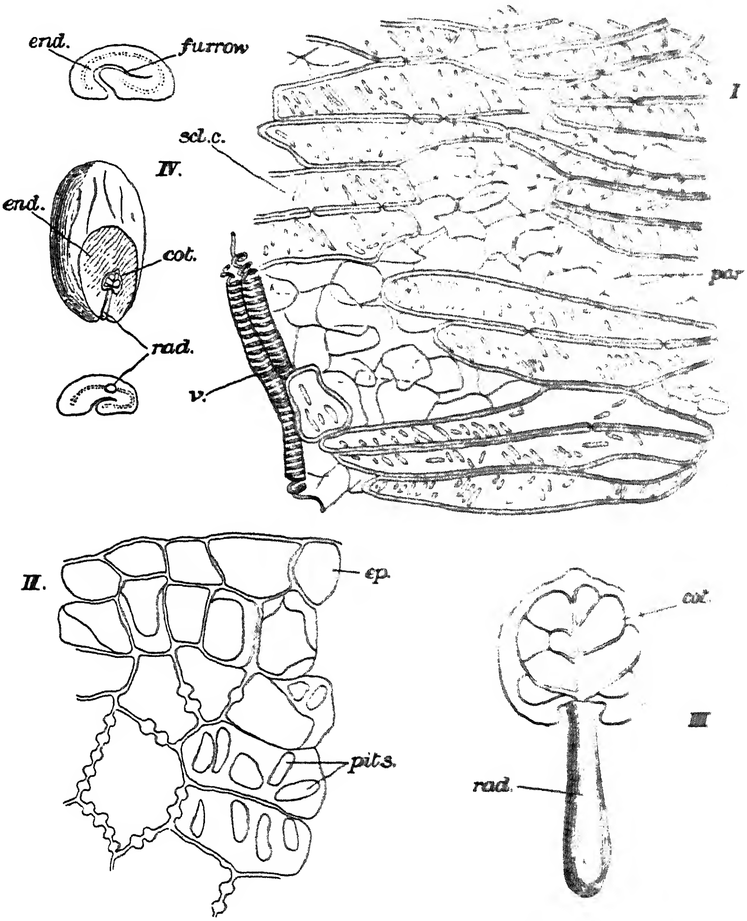

FIG. 124.—Coffee. I., portion of the seed coats, surface view; *par.*, collapsed parenchymatous tissue; *scl. c.*, sclerenchymatous cells; *v.*, vessel, ×220. II., transverse section of outer part of endosperm; *ep.*, epidermis, ×220. III., embryo of seed; *cot.*, cotyledon; *rad.*, radicle. IV., sections of seed; *cot.* cotyledon; *end.*, endosperm; *rad.*, radicle.

They vary exceedingly in size, but many range from 150 to 350 μ in length. They usually taper bluntly, but sometimes they are terminated by flat transverse walls. They are often arranged side by side with their long axes parallel, and bear numerous large oblique pits. The phloroglucin reaction shows that their walls are lignified.

Occasional fragments of small vessels derived from the raphe may also be found.

Examination of the Endosperm.—Cut transverse sections, and mount them in chloral hydrate. The epidermis and one or two layers immediately beneath it are composed of cells with evenly thickened walls; the rest of the endosperm consists of parenchymatous cells with thick walls and very large pits. The pits are so large that they form ovate spaces that may be as long as the cell is wide, or may not attain half that length. In section, the cell wall appears thick and beaded. The embryo can be easily dissected out from the soaked bean. The thick radicle and two small, cordate, leafy cotyledons consist of small, delicate, parenchymatous cells.

The chief cell contents are oil, proteid matter, and occasional small starch grains.

Examination of Ground Roasted Coffee

The tissues that have been observed suffer no material change by the process of roasting to which the beans are subjected in the preparation of coffee for table use, but the cell contents are partly altered.

Reduce a few roasted beans to a coarsely granular powder. Separate the fine particles by sifting; from some of the coarse ones prepare sections by softening them and fixing them in pith. The sections are very deeply coloured, but may be readily decolourised by a short maceration in solution of chlorinated soda; examine them in dilute glycerin, and compare with the sections of the bean.

The fine powder is also so deeply coloured as to require some preparation before examination.

Macerate a little in solution of chlorinated soda, separate by centrifugation or otherwise, and wash once with distilled water; examine in dilute glycerin. The cells of the endosperm are now very clear, and easily examined. The sclerenchymatous cells of the seed coat are also readily found; these are always present in genuine ground coffee. In the endosperm cells, oil globules can be detected.

Digestion with caustic potash may also be employed to remove the colouring matter, but it is not so successful as chlorinated soda.

Ground coffee consists principally of fragments of the endosperm, which have the characters above described. With these there are always associated portions of the seed coat which are characterised by the sclerenchymatous cells. The *débris* of the embryo are not easily found, and there are only very few small spiral vessels present.

Examination of Commercial Coffee

Separate some of the coarsest fragments by sifting, examine them with a lens, and pick out any that are of suspicious appearance; soften these, and prepare sections from them decolourise, if necessary, with chlorinated soda, and examine.

Decolourise the fine powder that passes through the sieve wash, and examine in dilute glycerin. Any foreign fragments are easily detected. For the characters of chicory see page 329.

Reduce a little of the sample to a moderately fine powder and examine this in the same way; no tissues foreign to genuine coffee should be present.

Throw a teaspoonful of the coffee on to the surface of water and watch it; genuine coffee floats, with the possible exception of a few highly roasted particles, while nearly all adulterants sink. Examine this sediment, first in water, for starch, and then, after decolorisation, for the tissues present

Grey Pea

Source.—The dried seed of *Pisum sativum*, Linn.

Preparation and Examination of Sections.—Prepare the seeds by soaking in water for twenty-four to forty-eight hours. Should the seed coats separate from the kernel, examine each separately. Embed in pith, and cut transverse sections Examine them in chloral hydrate. The following tissues can be observed.

(1) *Epidermis*, formed of palisade cells about 12 μ wide and 58 μ or more high. The walls are cellulose, and are strongly and irregularly thickened. The lumen of the cell narrows near

the centre, and expands again, towards the base, into a cavity with an irregular, wavy or jagged outline. The walls are traversed by pits, which appear in this section as longitudinal striations; they are better seen in a surface preparation.

(2) *Hypoderma*, consisting of a single layer of cells about 30 μ wide and the same height. These cells are constricted about the middle, and thus assume the shape of a dumb-bell or capstan. They are known as 'bearer' cells. Their walls are rather thick and not lignified; they are traversed by longitudinal pits, which often widen as the cell widens.

(3) *Parenchyma*, composed of six or eight rows of delicate, more or less collapsed, parenchymatous cells of moderate size.

If the seed is examined carefully there will be seen near the hilum a small lenticular patch of a whitish colour. Transverse sections through this patch show that here the epidermis consists of a double row of palisade cells (fig. 126, III. and IV.), and also that it is not continuous, but exhibits a narrow slit which runs along the oval patch, opening towards the seed coats. Below this slit and parallel to it, there is a collection of lignified, porous cells, which, in transverse section, is flask-shaped. These cells are regarded as tracheids, but their function is not accurately known; they are found in nearly all leguminous seeds. The tracheids are surrounded by three rows of delicate parenchymatous cells.

The presence of these tissues necessitates a considerable increase in the thickness of the seed coats in their neighbourhood. The hypoderma is replaced by about three rows of thick-walled, deeply pitted cells resembling ordinary sclerenchymatous cells, which pass into rounded, thick-walled cells with short projecting arms, forming a loose tissue with numerous air spaces.

Surface Preparations.—Separate the seed coats and macerate for twelve hours in cold 5 per cent. solution of potash; tease well out with the needles.

(1) *Epidermis.*—The cells are polygonal in surface view, and exhibit numerous pits, which appear as dark lines radiating from a small dark point (cell cavity).

(2) *Hypoderma.*—The cells are polygonal in outline and have pitted walls; on focussing down the constricted portion of the cell appears as a bright ring in the centre of the cell; this ring is traversed by pits, and similar pits extend from

the margin of the ring to the outer line This phenomenon is easily explained by a comparison with the section of the [illegible]

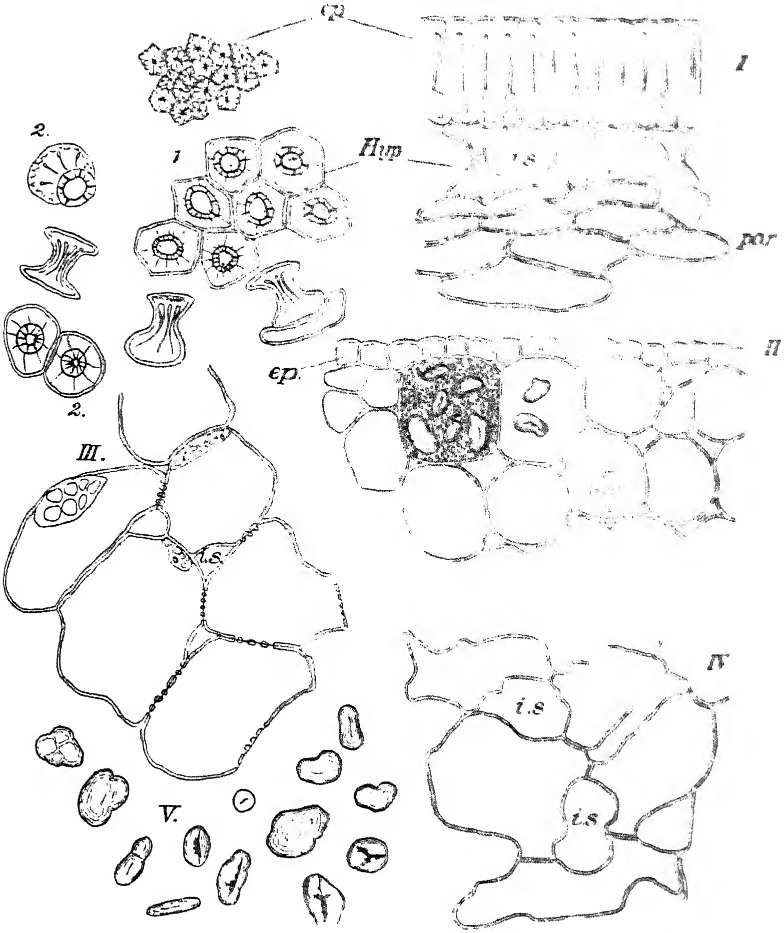

FIG. 125.—Pea. I., transverse section of seed coat, *ep*, epidermis; *Hyp*., hypoderma; *i.s*., intercellular space; *par*., parenchyma; 1, cells of hypoderma viewed from below; 2, viewed from above and from side. II., transverse section of outer portion of cotyledon; *ep*., epidermis. III. and IV., parenchyma of seed coat, surface view. V., starch. ×250.

(3) *Parenchyma*.—The cells are thin-walled, and exhibit intercellular spaces; near the cotyledons they are of considerable size.

Examination of the Cotyledons.—Proceed now to examine the cotyledons. Prepare transverse sections from a soaked seed, and examine in dilute glycerin.

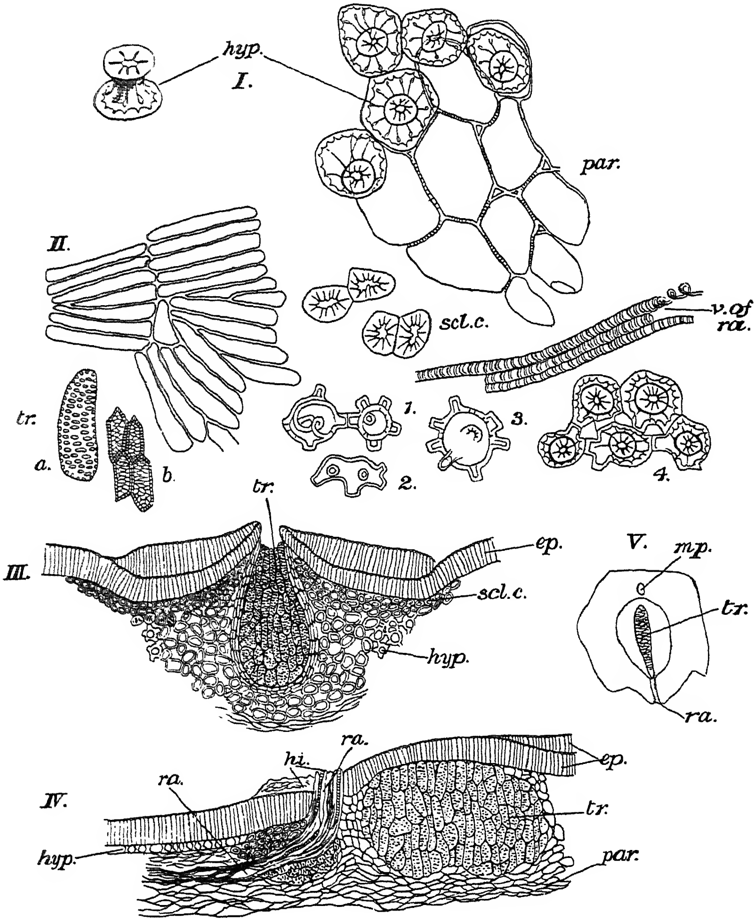

FIG. 126.—Pea. I., hypoderma viewed from above, with subjacent parenchyma. II., epidermis of cotyledon, surface view; 1, 2, 3, 4, modified epidermal cells from near the furrow. III., transverse section through the group of tracheids; *ep.*, epidermis; *hyp.*, modified hypoderma; *scl. c.*, sclerenchymatous cells of same; *tr.*, tracheids. IV., longitudinal section through the group of tracheids; *ep.*, epidermis; *hi.*, hilum; *hyp.*, hypoderma; *par.*, parenchyma; *ra.*, raphe; *tr.*, tracheids. V., lens view of group of tracheids. I. and II., ×250; III. and IV., ×50.

The epidermis consists of small cells wh[illegible] transverse section, and contain aleurone gr[illegible] Below the epidermis are large rounded parenchyma[illegible]

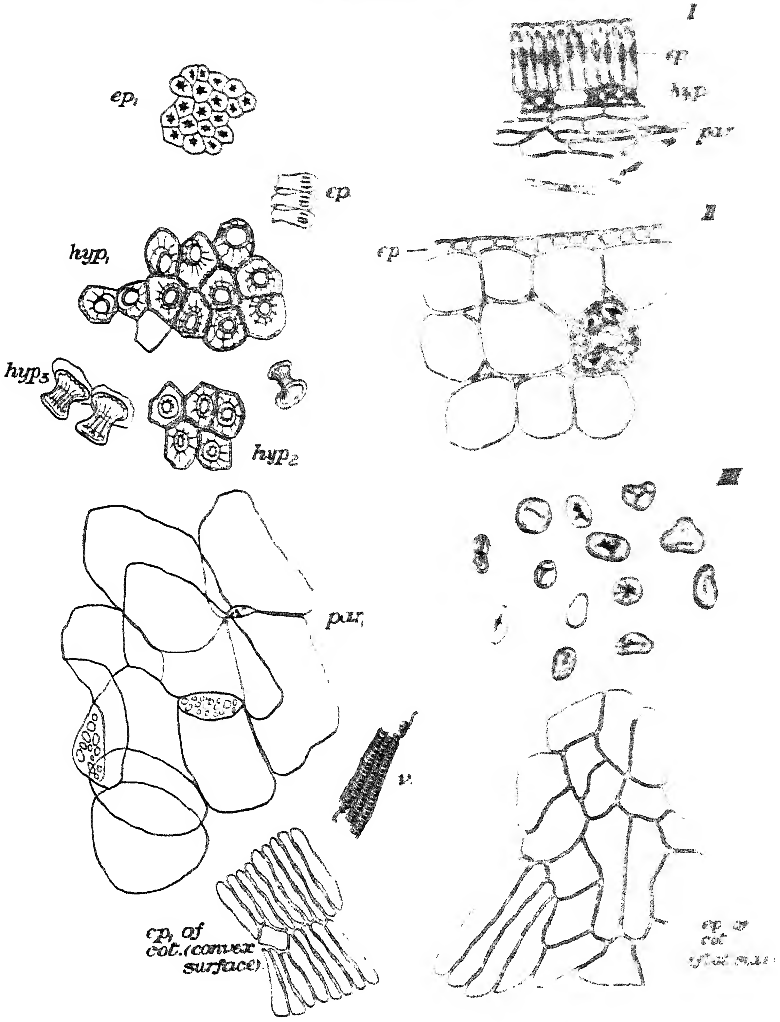

FIG. 127.—Lentil. I., transverse section of seed coat; ep., epidermis; hyp., hypoderma; par., parenchyma. II., transverse section of cotyledon; ep., epidermis. III., starch grains; ep., epidermal cells, after treatment with potash; ep1, epidermal cells, surface view; hyp1, hypoderma viewed from below; hyp2, the same from above; hyp3, hypodermal cells isolated by potash; par1, parenchyma of seed coat, surface view; v., vessels from raphe. All ×200.

with small intercellular spaces and pitted walls. They contain starch grains and innumerable small aleurone grains. In

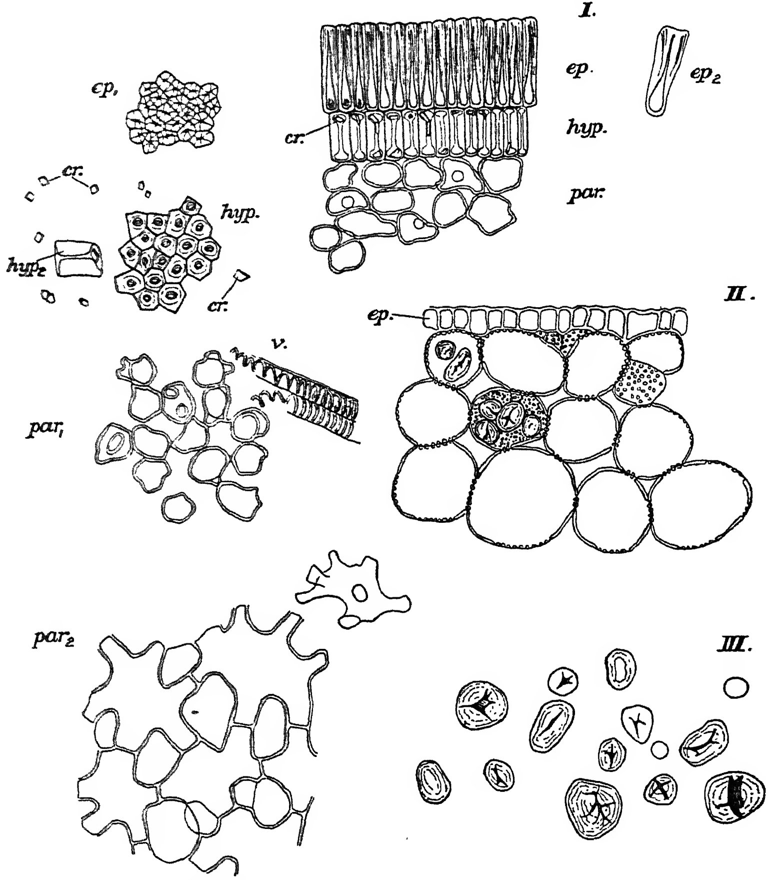

FIG. 128.—Haricot Bean. I., transverse section of seed coat; *cr.*, crystals of calcium oxalate; *hyp.*, hypoderma; *par.*, parenchyma. II., transverse section of cotyledon; *ep.*, epidermis. III., starch grains; *ep*$_1$, epidermis of seed coat, in surface view; *ep*$_2$, epidermal cell, isolated by potash; *hyp*$_1$, hypoderma, in surface view; *hyp*$_2$, hypodermal cell, isolated by potash; *par*$_1$, parenchyma of seed coat, surface view; *par*$_2$, inner layers of same. All ×200.

surface view the epidermal cells are seen to be narrow and elongated.

Examination of the Starch.—Examine some of the starch grains in water. They vary somewhat in shape, but many are oval-oblong, subreniform, or rounded, the majority showing remarkable enlargements at different points. Length about 20 to 40 μ.

	Bean	Pea	Lentil
(a) Palisade epidermis	30 to 60 μ long; not conical towards cuticle; wall smooth on interior; lumen wide near the inner wall, gradually or quickly contracting towards the outer wall	75 to 110 μ long (mostly 90 μ), not conical towards cuticle; inner wall irregular; lumen wide near the inner wall, contracting in the middle and widening again near the exterior	Up to 45 μ long, with a short conical projection towards the cuticle; inner wall smooth; lumen very wide, contracting towards the outer wall
(b) Hypoderma	Transverse section four-sided, without intercellular spaces (radial diameter 15 to 30 μ, transverse 15 to 25 μ), thickened at the sides and containing crystals of calcium oxalate (6 μ)	Delicate goblet, beaker, or dumb-bell shaped cells, inner wall rather broader than the outer; at the side large intercellular spaces (radial diameter 30 to 36 μ, transverse 36 to 45 μ) rather thick-walled, with cleft pores, but without crystals	Compressed dumb-bell or hourglass shaped, seldom elongated, often irregular, with intercellular spaces and cleft pores (radial diameter 9 to 24 μ, most 15 to 18 μ; transverse 15 to 30 μ), without crystals
(c) Parenchyma of cotyledons	Cells with thick walls and large pores, wall at least 5 μ thick and coarsely beaded in transverse section	Cells with moderately thick walls, pitted; wall at most 3 μ thick; cell wall in transverse section smooth or slightly beaded	Cells with thin walls, which are only slightly or indistinctly pitted in transverse section
(d) Starch	Grains up to 57 μ, the majority of regular elliptical, reniform, or bean-shaped, with long branching cleft through the hilum and very distinct striations	Grains 15 to 47 μ (or up to 51 μ), principally of irregular shape with rounded protuberances, together with reniform and bean-shaped grains, few being regularly elliptical; many without a fissure through the hilum; hilum and concentric striation visible	Grains 9 to 45 μ (most 20 to 40 μ) partly resembling bean and partly pea starch; many show concentric striations, but not so distinct as bean starch; many have no fissure, others a small unbranched fissure through the hilum

Examination of the Powder.—Mount in water, and examine the starch grains.

Mount in chloral hydrate, warm, and run in glycerin. The cells of the cotyledons are distinguishable everywhere. The epidermis is often in flakes, exhibiting its surface view. The hypoderma is also easily found, and, like the epidermis, exhibits its surface view, the cells being easily identified. The subjacent parenchyma can also be detected, but it is not shown very clearly. Here and there spiral vessels from the raphe may be found, but the tracheids from the neighbourhood of the hilum require diligent searching before they can be detected.

The structure of the pea may be taken as typical of leguminous seeds. These are characterised by the remarkable palisade epidermis, by the bearer cells, and by the group of tracheids near the hilum. Most of them also contain starch grains more or less closely resembling those of the pea or bean in general features. Leguminous flours are, therefore, easy of detection when mixed with cereal or other flours. Vogl[1] gives the foregoing useful table showing the chief points of distinction between the various leguminous flours. In order to facilitate comparison, illustrations of the structure of the lentil and haricot bean have been given.

Almond

Source.—The dried seed of *Prunus Amygdalus,* Stokes, var. *dulcis*, Baillon.

The almond may be taken as a type of the oily seeds which are much used as foods.

Examination of the Seed Coats.—Cut transverse sections of the seed coats and part of the cotyledon of a Valencia almond (without previous soaking) and transfer them to alcohol; mount one in glycerin and examine under the low power.

Observe the epidermis of the seed coats; it consists of a single layer of conspicuous, very large cells (giant cells) with brown, thickened walls interrupted here and there by rather smaller cells with thin colourless walls. Examine the giant cells carefully, using the high power when necessary. The cells vary much in size (up to 100 μ wide and 170 μ high); many of them have arched outer walls, others are nearly rectangular; the radial walls are pitted, especially towards

[1] *Nahrungs- und Genussmittel*, p. 166.

the base; the inner tangential walls are conspicuously pitted. These cells are very characteristic of the almond, although they vary somewhat in size in the different varieties. Occasionally brownish masses reacting for tannin, or small starch grains, may be found in them.

Below the epidermis is a layer of more or less collapsed parenchyma traversed by large fibrovascular bundles. These bundles are characterised by the presence of abundant, small, spiral vessels and crystals of calcium oxalate. Many of the cell walls are brown, and the cells sometimes contain brownish masses, or calcium oxalate in cluster crystals or in groups of several single crystals.

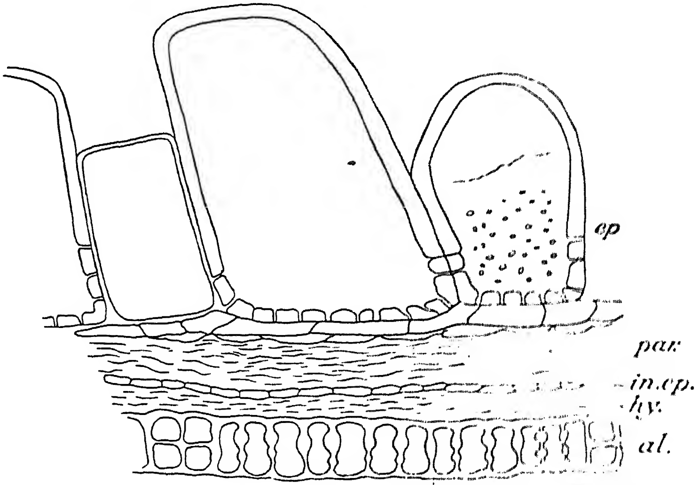

FIG. 129.—Almond, transverse section of seed coats. *al.*, aleurone layer; *ep.*, outer epidermis; *hy.*, hyaline layer (remains of endosperm); *in. ep.*, inner epidermis; *par.*, parenchyma of seed coats. × 240.

This tissue is also traversed by a single, tangential layer of small, narrow cells (about 5 μ wide, 15 μ long) constituting the inner epidermis of the seed coats. It is bounded by a conspicuous layer of aleurone cells (inner epidermis of the endosperm), the tissue between the two epidermises being the remains of the endosperm.

The cells of the aleurone layer are about 20 μ wide and 30 μ high; they have rather thick, distinctly beaded walls and contain proteid matter and oil.

Examine a section in chloral hydrate. The inner epidermis of the seed coat becomes more distinct; the walls of the aleurone cells swell.

Surface Preparations.—Warm a small fragment of the seed coats in solution of chloral hydrate. Examine under the low power. The epidermal cells are polygonal in outline and the inner tangential walls are coarsely reticulated.

Scrape the surface of a dry almond with the sharp edge of a glover's needle. Moisten with alcohol the powder thus obtained and examine in solution of chloral hydrate. The giant cells of the epidermis are well seen.

Separate the skin of an almond after soaking in hot water. Scrape off from the inner surface the aleurone layer and inner portions of the seed coat and mount in glycerin. The aleurone layer and the inner epidermis of the seed coat can easily be seen and distinguished by the thickness of the cell wall.

Examination of the Cotyledons.—Cut transverse sections. Defat them in ether-alcohol. Examine one in solution of chloral hydrate. The tissue consists of rather large (30 to 60 μ), rounded or polygonal, thin-walled cells; the epidermal cells are rectangular and much smaller.

Examine the contents of the parenchymatous cells very carefully.

Transfer two or three of the thinnest sections to a slide; add a small drop of a saturated, aqueous solution of picric acid; after two or three minutes remove the excess of solution with filter paper; add a small drop of glycerin and examine. The cells contain numerous small (3 to 5 μ) aleurone grains, often accompanied by a single large one (10 to 15 μ); they are stained yellow. The large aleurone grain usually contains a small, granular rosette of calcium oxalate, often with a minute but conspicuous central point. Smaller aleurone grains containing crystalloids may also, with careful searching, be found. The smallest of the grains contain minute globoids.

Examine a section stained with picric acid in water or dilute glycerin. Irrigate gently with a 0·3 per cent. solution of caustic potash. The ground substance and crystalloids dissolve, but the calcium oxalate and the globoids remain. The minute rosettes of calcium oxalate, with their distinct central points, are characteristic.

Examination of Almond Meal.—Almond meal is almond press-cake reduced to powder. It may be examined and identified as follows:

(i) Moisten a little with alcohol, add solution of chloral hydrate, and examine after a few minutes. Fragments of the giant cells of the epidermis are conspicuous by their golden yellow colour and thickened, partly pitted walls. Portions of the tissue of the cotyledons, recognisable by the thin walls of the cells, are easily found, as well as small groups of a few cells, or even parts of the cell walls only. Pieces of the aleurone layer in surface view are not difficult to find and identify by their thick walls.

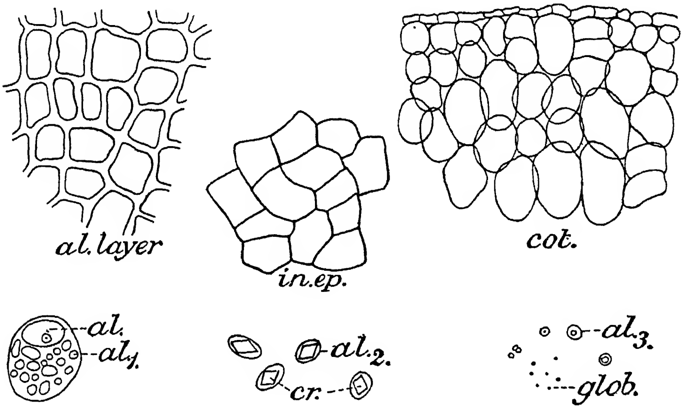

FIG. 130.—Almond. *al. layer*, aleurone layer in surface view; *cot.*, cotyledon, in transverse section; *in. ep.*, inner epidermis of seed coat; *al.*, large aleurone grain; *al.*$_1$, small aleurone grain. All × 240. *al.*$_2$, *al.*$_3$, small aleurone grains; *cr.*, crystalloids; *glob.*, globoids. × 480

(ii) Moisten a little with alcohol, add glycerin, and examine after an hour or two. The aleurone grains are distinct; portions of the tissue of the cotyledons, with the cells packed with aleurone grains, may also be found.

(iii) Moisten with alcohol, add picric acid and then glycerin. The small aleurone grains are abundant. Large grains may also be found as well as grains with crystalloids, but these are less numerous.

(iv) Mount a little in dilute solution of potash. The characteristic calcium oxalate rosettes are abundant.

The ground kernels are largely used as a food for diabetic patients. It may be conveniently examined by defatting it with ether followed by ether-alcohol. The residue obtained by pouring off the ether-alcohol solution may be treated as above (without drying). As the seed coats are almost entirely absent, reliance must be placed on the characters of the aleurone grains and the parenchyma of the cotyledons.

Walnut

Source.—The seed of *Juglans regia,* Linn.

Examination.—The dried (shelled) walnuts of commerce may be examined as directed for almonds. The chief distinctive features are:

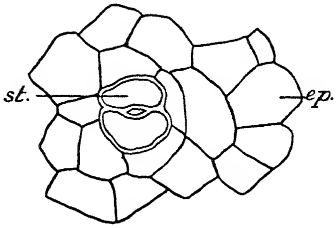

FIG. 131.—Walnut kernel, epidermis, in dilute glycerin, after warming. *ep.*, epidermal cell; *st.*, stoma. × 240.

(*a*) The epidermis of the brown seed coat. This consists of thin-walled polygonal cells which, in transverse section, are radially elongated. It exhibits large, characteristic stomata, which are often considerably distorted; the epidermal cells contain transparent amorphous masses which slowly dissolve in water, yielding a solution which gives an intense blue-black precipitate with ferric chloride.

(*b*) The contents of the cells of the cotyledons; these consist of oil and very small rounded or oval aleurone grains containing minute globoids, but no calcium oxalate.

Pine Kernels

Source.—The kernels of the seeds of *Pinus Pinea*, Linn. and *P. Cembra*, Linn.

Examination.—They may be examined as indicated for almonds, making, in addition, surface sections of the kernels.

The chief distinctive characters are:—

(*a*) The epidermis of the endosperm; this consists of rather large, thick-walled polygonal cells.

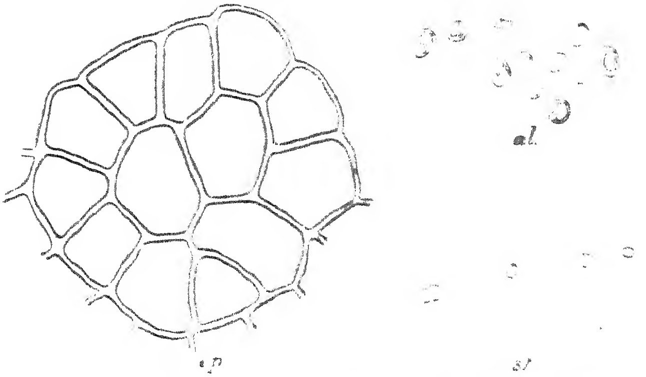

FIG. 132.—Pine kernel: *ep*, epidermis, [illegible]; *al*, aleurone grains; *st*, starch, [illegible].

(*b*) The contents of the cells of the endosperm and embryo. These consist of oil, together with abundant aleurone grains, and numerous minute starch grains. The starch grains may be readily detected by chloral iodine. Some of the aleurone grains contain a globoid and crystalloid, others a single globoid, others again numerous minute globoids.

Oil Cake

The cake obtained by submitting seeds containing fixed oil to strong pressure, in order to extract the greater part of the oil contained in them, is largely used as cattle-food and the determination of the identity and purity of the material employed is frequently a matter of importance. Occasionally the seeds are freed from their integuments before they are crushed and pressed, but more commonly this preliminary treatment is omitted. Sometimes the oil is removed from the crushed seed by means of a volatile solvent, and the residue, freed from the solvent, is pressed into cakes.

Apart from the preliminary inspection of the cake, its colour, fracture, odour when mixed with water, &c., several methods have been proposed for the microscopical examination of oil cake. The following (Collin and Perrot's method) is the most important:

Disintegrate with the aid of a glass rod one or two grammes of the bulked cake and diffuse it through 100 c.c. of warm water contained in a small porcelain dish. Allow the fragments to deposit and pour off the supernatant liquid, collecting separately the last portions in which a little pulverulent matter remains suspended. Set this turbid liquid aside to deposit, and examine the sediment for fragments of the cotyledons and endosperm as well as for starch and aleurone grains. The aleurone grains may also be examined in a little of the fine powder sifted from the crushed cake.

To the contents of the dish add 80 c.c. of water and a few drops of a concentrated (25 per cent.) solution of potassium hydroxide. Boil for ten minutes and note the colour of the supernatant liquid, as well as of the deposit. Wash the deposit several times with distilled water, allowing it to settle for ten minutes each time, or using a centrifuge. When the washings are colourless pour off the liquid, leaving in the dish a depth of about 0·5 to 1 cm. Holding the dish in the hollow of the hand, impart to it a rotary motion, so as to separate the elements present according to their specific gravity. Incline the dish now at an angle of about 45° in order to expose the elements at the bottom of the dish. Remove portions of the various layers in the dish with a brush and transfer them to watch-glasses standing on white paper. Examine first the fragments from the bottom layer. The colour is not entirely to be relied upon as a guide, as the seed coats of the same seed may vary considerably. From large particles transverse sections may be made. Others may be disintegrated by means of dissecting needles or a scalpel. In case of doubt

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[1] *Les Residues Industriels*, Paris, 1904.

further pulverisation, by means of powdered glass if necessary, should be resorted to.

Next examine the fragments from the lighter greyish layer, which contains chiefly colourless integuments and fragments of the cotyledons. These may with a little practice be distinguished from one another by the naked eye as the former occur as membranous plates, the latter as solid particles.

The deposit obtained after boiling with dilute alkali may also be bleached by solution of chlorinated soda, washed and stained, if necessary, by any appropriate method (see p. [illegible]).

Identification of a Powder as that of a Seed

Among the characters that distinguish powdered seeds from other powders, the aleurone grains occupy a position of great importance. These grains are found in ripe seeds only, and, if found, point definitely to the presence of a seed. Large and well-developed aleurone grains are comparatively easy to detect and examine, but that is by no means the case with those of very small size.

The presence of much reserve starch, fixed oil, or fat also indicates a seed powder, and the same may be said of reserve cellulose, which generally occurs in the form of strongly thickened walls of the endosperm.

Notable quantities of vascular tissue are usually absent, the only vessels found in seed powders being those derived from the raphe, and these are commonly very small. Sclerenchymatous fibres (bast fibres) are also absent, and the powder should be free from chlorophyll, from epidermis with stomata, and from cork tissue.

SECTION XI

FRUITS

INTRODUCTION

Fruit is the term applied by botanists to the whole product of the development of the gynœceum as a result of fertilisation. Sometimes other parts of the flower in addition to the gynœceum participate in the production of the fruit, which is then termed spurious.

In dealing, therefore, with the anatomy of the fruits, it is evident that, in addition to such tissues as form the seed coats and kernel of the seed, the structure of the tissues that have been developed from the carpellary walls of the ovary must be studied, and also, if the fruit examined be a spurious one, the structure of the tissues that are derived from such other parts of the flower as combine to produce the organ under examination.

The seeds that are developed in dehiscent fruits have, as a rule, to remain for some time exposed to the inclemencies of the weather, and in such seeds it is natural to expect a considerable development of the seed coats into more or less resistent, protective coverings. The seeds described in the preceding section were seeds of dehiscent fruits, and, as a general rule, it will be found desirable to remove the seeds of such fruits from the pericarp and examine each organ separately. But the seeds contained in indehiscent fruits usually rely, more or less completely, upon the protection afforded to them by the tissues of the enveloping pericarp, and in such cases it is frequently found that the seed coats are reduced to a very narrow layer composed of but few rows of cells, which have often so completely collapsed as to render their structure difficult of examination. Pepper and cubebs, the umbelliferous fruits, and many others furnish examples of this. So also do the graminaceous fruits, which, from the large extent to which they serve as foodstuffs, are especially important. Among the latter barley may particularly be noticed, inasmuch as, in addition to the pericarp and seed coats, the tissues of the paleæ, which remain permanently attached to the pericarp and hence form part of the so-called fruit, have to be considered; indeed, as far as its structure is concerned, this fruit is one of the most complex with which the histologist has to deal.

The great diversity in structure exhibited by the official fruits results in the presentation of a number of diagnostic characters that may be utilised in the identification of the drug or its powder, but it also brings with it the absence of any general structural plan such as is observed in leaves, stems, and barks.

The epidermis is in most cases well preserved, and the form and disposition of its cells may be useful. Stomata are generally recognisable, though they have often undergone considerable change. Like the leaves, the epidermis of the pericarp may bear hairs, which then offer valuable diagnostic features.

The tissue subjacent to the epidermis is, in many cases, a parenchyma traversed by fibrovascular bundles, and comparable with the mesophyll of the leaf. In this tissue, sclerenchymatous cells or groups of cells are often to be found (pepper, pimento, cubeb). In such cases the characters of these cells must be accurately determined. Oil cells (pepper, cubeb), oil glands (pimento), oil ducts (most umbelliferous fruits), lactiferous vessels (poppy capsules)—in short, secretory tissue of various kinds—may be present, all of which are of high importance. Calcium oxalate, starch, and other cell contents contribute their share to the characteristics of the drug.

On the inside, the pericarp is bounded by an epidermis which, like the outer epidermis, may present particular features.

Diagnostic characters of fruits may be sought for in the following points (in addition, of course, to those furnished by the seed, which have been dealt with in the preceding section).

(*a*) The outer epidermis, more particularly the hairs, if present.

(*b*) The sclerenchymatous tissue in the subjacent parenchyma; the shape of the cells, their size, character of the pits, &c.

(*c*) The presence and character of secretory tissue of any kind.
(*d*) The cell contents, more particularly calcium oxalate and starch.
(*e*) The characters of the inner epidermis.

Cardamom Fruit

Source.—The fruit of *Elettaria Cardamomum*, Maton.

Examination of the Seeds

Preparation.—Separate from Mysore Cardamom fruits some seeds that are full grown and nearly, but not quite, ripe; these may be recognised by their colour, which is not quite so dark as that of the ripe seeds. The structure of unripe seeds is similar to that of ripe, but the seeds are easier to cut before they are quite ripe.

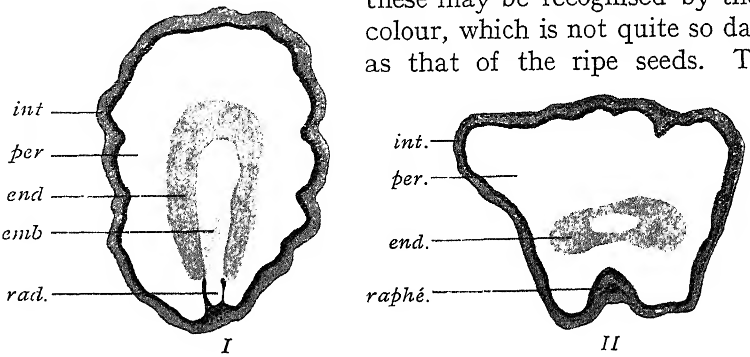

FIG. 133.—Cardamom Fruit. I, longitudinal section of seed; II, transverse section of the same; *emb.*, embryo; *end.*, endosperm; *int.*, integuments; *per.*, perisperm; *rad.*, radicle. × 14.

Soak them in water for several days; should they become too soft, harden them a little in spirit.

After soaking in water a semi-transparent membranous coat, which was previously scarcely to be detected, becomes visible; this is the arillus. It is attached to the micropylar extremity of the seed.

Remove the arillus, and cut the seed longitudinally in half. Examine with a lens, and observe in the centre the small elongated-oval embryo, surrounded by a scanty, yellowish endosperm, which is itself enclosed in a more abundant whitish perisperm, the whole being surrounded by a dark narrow seed coat. Cut another seed transversely, and identify the same parts in transverse section.

Arillus.—Soak a seed for a few minutes in water, and strip the arillus from it; mount and examine in water or dilute glycerin. It is composed of several layers of narrow elongated cells, with delicate walls, which are often not very easy to see; they contain minute globules of oil and occasional small rosettes of calcium oxalate.

Sections of Seed Coats.—Fix a seed in pith (or cork) and cut transverse sections; examine in water or dilute glycerin.

All the cells of the perisperm are filled with dense granular masses of starch, while those of the endosperm and embryo contain hyaline substances; these tissues and their contents will be subsequently more closely examined.

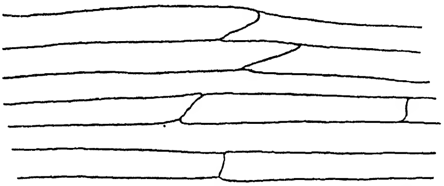

FIG. 134.—Cardamom Fruit. Arillus of seed. × 200. (The cell contents are not shown.)

Mount another section in solution of potash; warm gently until the starch is gelatinised, but do not boil.

The outermost layer of cells is the epidermis of the seed coat. The cells appear small and nearly quadratic in section. In the depressions of the seed they are larger than they are in the elevated parts.

Examine in glycerin a section not previously warmed with potash. The cells are thickened on the outer and inner walls; it is evident, on comparison, that warming with potash has made them swell.

Next to the epidermis observe, in the potash preparation, very flat collapsed cells, often coloured brown by the action of the alkali (fig. 135, p_1); these separate the epidermis from a single row of large rectangular cells with thin strongly refractive walls. The brown cells are often rather difficult to detect, but they may generally be found by carefully examining the line that separates the rectangular cells from the epidermis. They constitute the second layer of the seed coat.

The large rectangular cells just referred to constitute the third layer. They contain volatile oil, but it is not often that the oil

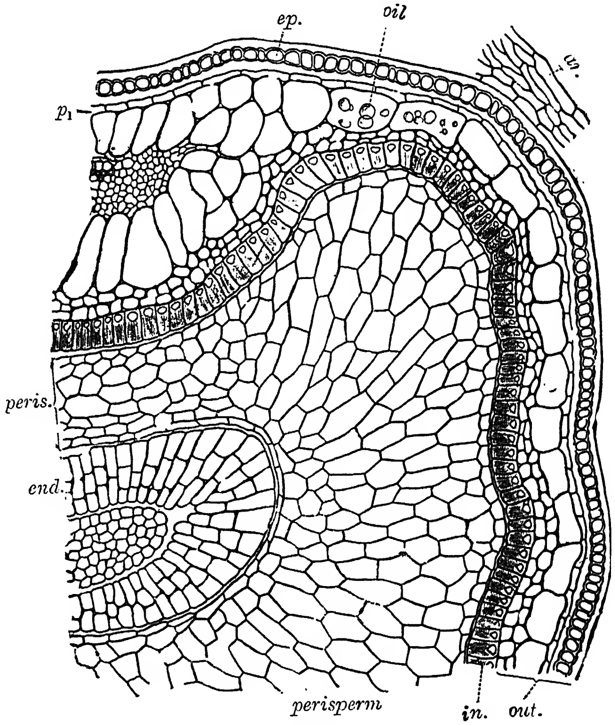

FIG. 135.—Cardamom Fruit, transverse section of seed. *ar.*, arillus; *end.*, endosperm, enclosing the embryo; *ep.*, epidermis; *in.*, inner integument; *oil*, oil cells with globules of oil; *out.*, outer integument; p_1, subepidermal parenchyma. (After Tschirch and Oesterle.)

can be seen in them, especially if the sections are thin. Its presence can, however, be easily demonstrated (see below).

Following upon the oil cells is a confused narrow layer of tissue which may be shown by the examination of earlier stages of development to be the remains of a single or double layer of cells, but its structure cannot always be distinguished in a transverse section. This is the fourth layer.

Next to the collapsed layer is a very conspicuous single row of brown or yellowish-brown cells. They are rectangular, radially elongated, and about 40 μ long and 20 μ wide. The radial and inner tangential walls are so strongly thickened that the lumen is small and funnel-shaped, the mouth of the funnel being directed outwards. The walls are faintly striated. This, the fifth layer, is the inner integument of the seed.

Within the inner integument is the perisperm, in the cells of which the calcium oxalate crystals are now distinctly visible ; sometimes there is only one crystal in each cell, sometimes there are three or four.

Take another section and boil very gently in solution of potash for a few seconds. Examine the sclerenchymatous cells of the inner integument, and observe in the lumen of each cell, nearly filling it, a small rounded or subconical body with a granular surface. These have been carefully examined and tested in various ways ; they appear to be nodules of silica.

Warm another section in chloral hydrate, cool, and examine ; the calcium oxalate crystals in the cells of the perisperm are very distinct.

Surface Preparations of Seed Coats.—Prepare surface sections from a seed ; the sections are apt to be difficult of interpretation, owing to the rugose surface of the seed. Examine them in succession, mounting in chloral hydrate, and paying, as usual, particular attention to the order in which the various layers of cells are arranged. Care must be taken to keep the outer surface uppermost.

The epidermis is easily identified by its very long, narrow cells ; the width of the cells should correspond to the width of the epidermal cells as seen in the transverse section. The oil cells (third layer) are conspicuous by reason of their large size and thin, highly refractive walls, but the cells of layer, which lies between the epidermis and the oil cells, are inconspicuous. They can, however, with care also be distinguished ; their long axes cross the long axes of the epidermal cells at right angles.

The layer of collapsed cells which follows next is very difficult to distinguish in surface preparations, but the sclerenchymatous cells are, again, easy of observation by reason of their colour, which varies from brownish-yellow to dark brown according to the degree of ripeness of the seed. In surface view the cells are small (about 15 to 25 μ), polygonal, and exhibit small cavities; their appearance, therefore, is quite different from that exhibited by the transverse section.

Within the sclerenchymatous layer the thin-walled cells of the perisperm, crowded with minute starch grains, occur.

Maceration Preparations of Seed Coats.—Prepare next maceration preparations of seed coats.

Digest a few seeds for an hour in a water-bath with 5 per cent. solution of potash; wash with distilled water; tease out, and break up the seed coats, as thoroughly as possible.

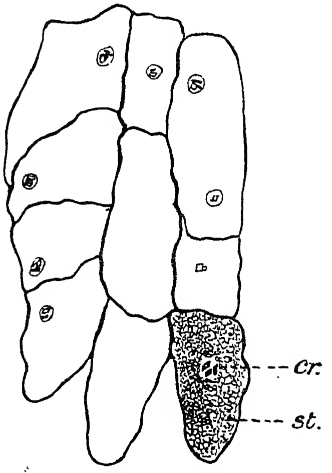

FIG. 136.—Cardamom Fruit, portion of perisperm. *cr.*, crystals of calcium oxalate; *st.*, starch (represented in one cell only). × 240.

The epidermis is generally easily found (fig. 137, *ep.*); it occurs mostly in flakes, but some of the cells will probably have separated from one another. Here and there delicate thin-walled cells can be seen; they are much shorter, but as wide as or rather wider than the epidermal cells, which they cross at right angles. These are the cells of the second layer, and it is in this preparation that they can best be seen (fig. 137, *par.*$_1$).

The oil cells are very conspicuous; they are large thin-walled cells of rectangular shape, without intercellular spaces; each one contains a large globule of oil.

The fourth layer, which follows upon the oil cells, is with difficulty to be found in the potash preparation.

The sclerenchymatous layer is very much darkened in colour by the action of the alkali, so that this layer from ripe seeds is almost black, and shows little or no structure; from unripe seeds it is reddish-brown, and the nodules of silica can be seen if the light is sufficiently powerful. The fourth layer often adheres to the sclerenchyma, and can best be found by separating the fragments of the latter and breaking them up as completely as possible. The cells of which the layer consists are rounded or polygonal, nearly isodiametric, and have thin delicate walls.

Sections of the Kernel.—Having now thoroughly examined the seed coats, the student may proceed to the kernel, which consists of perisperm, endosperm and embryo.

Cut a few transverse sections and examine in water.

The cells of the perisperm are packed with minute starch grains ; in the centre of each cell there is a cavity in which one or more minute crystals of calcium oxalate may be seen. Examine the starch grains ; they are very small (1 to 3 μ), rounded or angular, and often translucent in the centre.

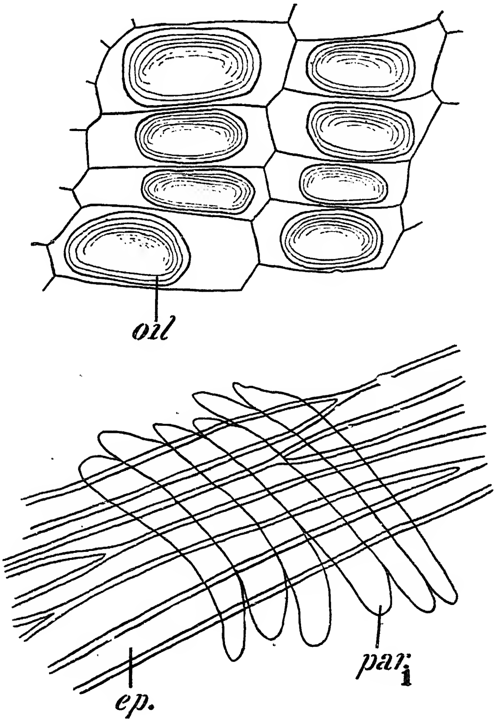

FIG. 137.—Cardamom Fruit, elements of the seed. *ep.*, epidermis ; *par.*$_1$, subjacent parenchyma ; *oil*, oil cells ; isolated by maceration with potash. × 240.

Next mount one or two sections in chloral hydrate, warm until the starch is gelatinised, cool, add a drop of glycerin, and examine.

The walls of the cells can be distinctly seen ; they are thin and often wavy; the calcium oxalate crystals are now very distinct.

The endosperm and embryo are best examined as follows:

Mount a transverse section of the seed in chloral iodine; observe that the starch contained in the cells of the perisperm stains blue, but the contents of the endosperm assume a distinct yellow colour, those of the embryo being less deeply coloured.

Mount another section in chlorzinciodine; the very delicate cell walls of the endosperm can be seen, if carefully examined.

Mount another section in water or chloral hydrate; oily globules exude from the cells of the embryo. If water is used the globules may be stained in the usual way.

Mount a defatted section in picric acid, as directed for mustard seed; observe the cells of the embryo; they are small, and filled with small aleurone grains, which stain with picric acid; the cell contents of the endosperm also stain.

The student will have now thoroughly examined all parts of the seed, and may proceed to identify them in the powder.

Examination of Powdered Seeds

(1) Mount a little powder in water or dilute glycerin, as usual, and examine. Very conspicuous are the perisperm cells packed with starch grains, and containing calcium oxalate crystals (better seen after gelatinisation of the starch). Irrigate with dilute solution of iodine; the starch turns blue.

(2) Mount a little in chloral iodine; the starch in the cells of the perisperm assumes a blue colour, but the cells of the endosperm and embryo are coloured yellow.

(3) Mount a fresh portion in saturated solution of picric acid, as directed for mustard seed; the fragments of endosperm and embryo are stained deep yellow; they may be made more conspicuous by warming until the starch in the cells of the perisperm is gelatinised. With care, the cell walls can be detected, especially after the addition of a little glycerin.

(4) Mount a little of the powder in Fehling's solution, heat to boiling, and cool; the fragments of endosperm are coloured violet (Meyer).

(5) Mount a little in chloral hydrate, as usual, warm until

the liquid boils, cool, and examine. In this preparation there can be found without difficulty—

(*a*) *Pieces of the Epidermis*; the epidermal cells are easily recognised by their moderately thick, straight or slightly curved walls, and more or less pointed ends. They are often crossed at right angles by the cells of the second layer; the latter are shorter and have thinner walls, but are of about the same width.

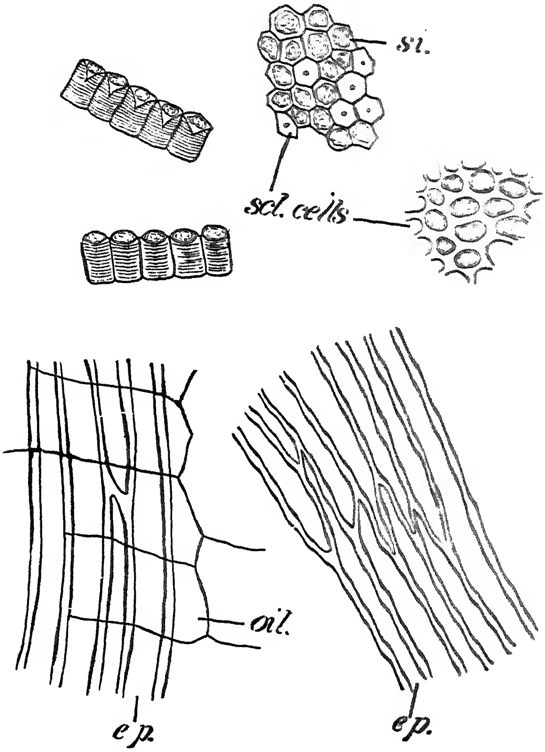

FIG. 138.—Cardamom Fruit. Characteristic elements of the seed coats, from the powder. *ep.*, epidermis; *oil*, oil cells, broken and free from oil; *scl. cells*, sclerenchymatous cells of inner integument; on the left in section; on the right in surface view from below (upper figure) and from above (lower figure); *si.*, nodule of silica. × 240.

(*b*) *Fragments of the Sclerenchymatous Layer*; these are very conspicuous by reason of their colour, which varies from yellowish in unripe to dark reddish-brown in ripe seeds. The surface, viewed from above, exhibits rather large cell cavities, in many of which the nodule of silica can be seen, the dark colour beneath the nodule being due to the thickened and coloured walls of the cell (compare fig. 138). Viewed from below, the polygonal outlines of the cells are distinct, and the cavities small.

(*c*) *Portions of the Perisperm*; the cells are readily identified by their size, by their moderately thick, pitted walls, and by the crystals of calcium oxalate which they contain.

Less easily found and identified are:

(*d*) *The narrow, elongated, thin-walled cells of the arillus.*

(*e*) *Portions of the endosperm and embryo*; the cells have thin walls, not pitted, and do not contain calcium oxalate crystals.

(*f*) *Débris of the oil cells*; these are mostly broken, and free from oil.

Examination of Pericarp

Sections.—Proceed next to the examination of the pericarp. Separate some pericarps from the seeds, and soften them by exposing them to a moist atmosphere for twelve hours. Cut transverse sections, place them for a moment in alcohol, transfer to water, and finally mount in dilute glycerin.

The section consists of parenchymatous tissue traversed by numerous fibrovascular bundles; it is bounded on the outer side by an epidermis consisting of small flattened cells, on the inner side by an epidermis, the cells of which are often so collapsed as to show little structure. The cells of the parenchyma

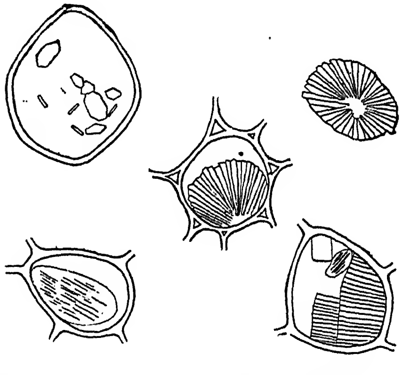

FIG. 139.—Cardamom Fruit. Calcium oxalate crystals in the parenchymatous cells of the pericarp. × 240.

are large, and have thin walls; most of them are empty, but some contain several single crystals of calcium oxalate. Here and there irregular sphærocrystalline masses of calcium oxalate

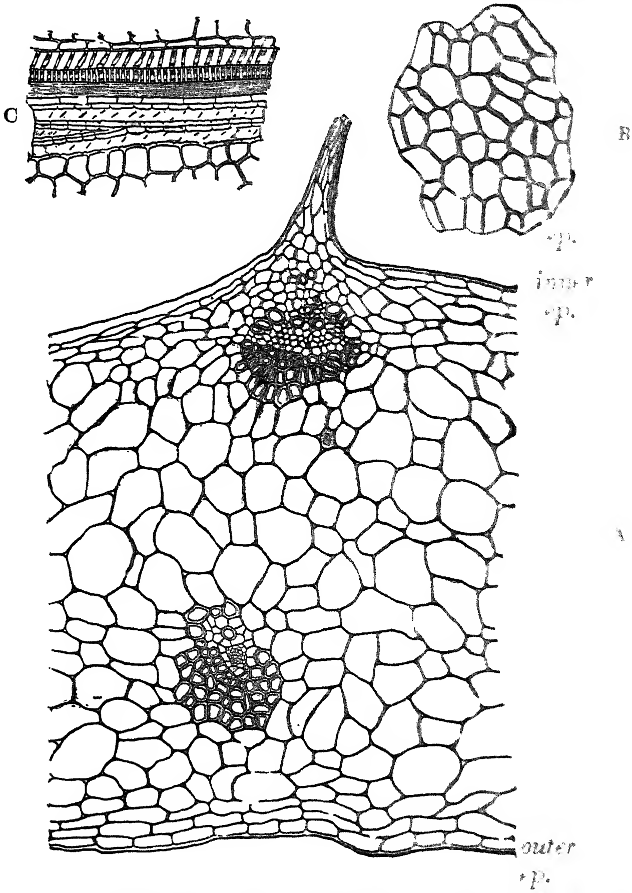

FIG. 140.—Cardamom Fruit. Structure of the pericarp. *A*, transverse section, with projecting carpellary wall, showing two fibrovascular bundles with supporting sclerenchymatous tissue; *B*, outer epidermis of pericarp, surface view; *C*, longitudinal section of bundle. (Tschirch and Oesterle.)

can be detected; they are rather irregular in distribution, and are best seen in sections taken from the inner surface; under the polariser they are easily detected. This tissue also contains scattered, rounded or oval cells, filled with a yellow or brownish resin.

The fibrovascular bundles consist of a few small spiral vessels and a scanty bast, protected (or surrounded) by an abundant crescent (or circle) of sclerenchymatous tissue. The elements of the latter have large cavities and moderately thick walls.

Maceration Preparations.—Macerate some fragments of the pericarp in potassium chlorate and nitric acid; wash, and tease out the sclerenchymatous cells and fibres. They vary considerably in length, in diameter, and in shape. They range from 10 to 1,000 μ in length, but average about 600 μ in length and 30 μ in width. The ends are rounded or blunt, not sharply pointed, and the walls are not very thick. They often have a wavy outline, due to the pressure of neighbouring cells. The pits appear, after treatment with the oxidising mixture, to be slits arranged in a left ascending spiral.

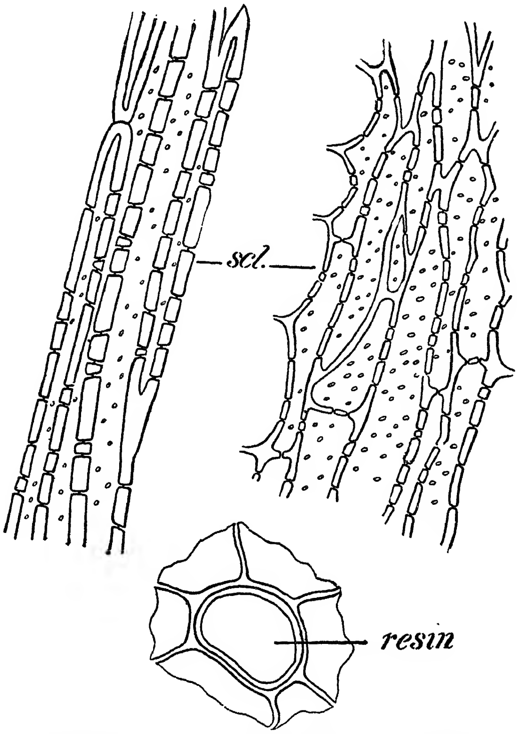

FIG. 141.—Cardamom Fruit. Sclerenchymatous cells and resin cell from the pericarp. × 240.

Examination of Powdered Fruit

The following examination should reveal the tissues and cell contents of the pericarp, in addition to those derived from the seed:

(*a*) In water or dilute glycerin:—large, empty, thin-walled parenchymatous cells, with an occasional cell filled with yellow or brown resin; groups of lignified cells and fibres, which may be distinguished from epidermal cells by the lignification of the walls and by the conspicuous pits.

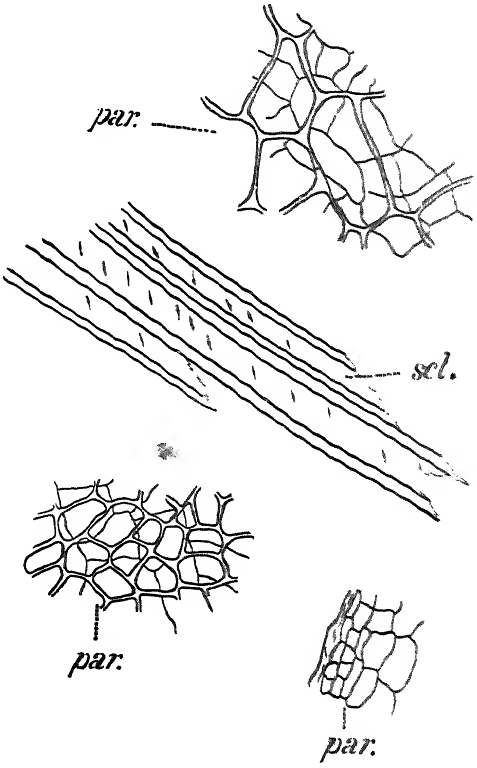

FIG. 142.—Cardamom Fruit, fragments from the powdered pericarp; *par.*, parenchymatous tissue; *scl.*, sclerenchymatous fibres. × 240.

(*b*) In chloral hydrate the fibres are well seen, and the radiating groups of calcium oxalate crystals, characteristic of the pericarp, may also be found.

(*c*) Macerate 0·5 gramme of the powdered fruit with 5 c.c. of water, 10 c.c. of nitric acid, and 1 gramme of potassium chlorate in a water-bath, until chlorine is evolved and the powder bleached; the starch and other cell contents should be entirely destroyed, the cellulose partly so, but the sclerenchymatous tissue should not be vigorously attacked. Separate the cell *débris* by a centrifuge, wash, and examine in water. Particularly distinct in this preparation are the nodules of silica from the inner integument of the seed. They appear as small, oval or rounded masses with a granular surface. The inner integument itself is also well seen, as the colour is partly discharged and the nodules of silica are very conspicuous. The fibres from the pericarp are very distinct, and often show spiral disintegration.

Colocynth Fruit

Source.—The fruit of *Citrullus Colocynthis*, Schrader. The fruits, which are more or less completely freed from the outer rind, may be divided into the following parts for examination:

(*a*) The rind. (*b*) The pulp. (*c*) The seed.

Examination of the Rind

Cut from a colocynth fruit one or two of the fragments of rind that are often left adhering to it. Expose them to a moist atmosphere for a few hours, and cut transverse sections; allow these to remain in alcohol as long as possible. Mount one in dilute glycerin, or in chloral hydrate. The section shows an epidermis consisting of a single row of radially elongated cells. These cells are about 15 μ wide and 20 to 25 μ high; the radial walls are strongly but not uniformly thickened, being thickest near the middle, tapering slightly towards the outside, but abruptly narrowing towards the inside. The outer tangential wall is cuticularised, and so are the radial walls, with the exception in each case of an inner layer of cellulose lining the cell. In surface sections the cells are polygonal, small, and rather thick-walled. Scattered over the surface of the fruit are large depressed stomata surrounded by thin-walled cells; the stomata may also be observed in transverse sections.

Following upon the epidermis is a layer about 150 μ wide, consisting of some fifteen rows of thin-walled, tangentially elongated, parenchymatous cells with pitted walls.

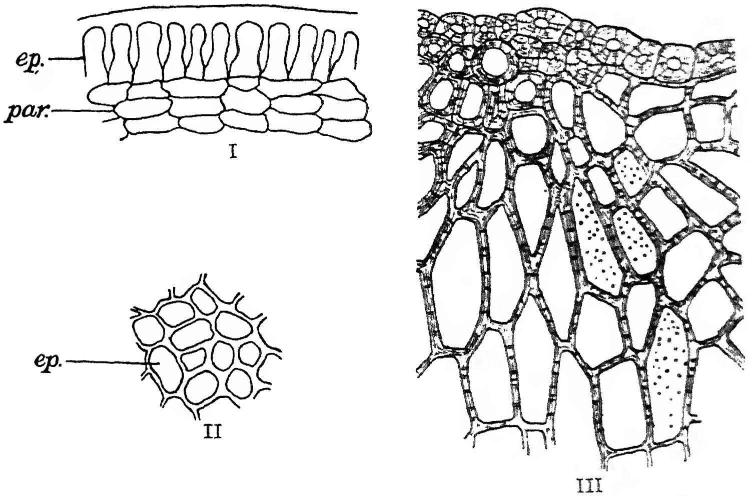

Fig. 143.—Colocynth Fruit. *I*, transverse section of outer portion of rind; *ep.*, epidermis; *par.*, subjacent parenchyma. *II*, epidermis in surface view. *III*, transverse section of sclerenchyma lying below the parenchymatous layer. × 240.

Next to this is a layer of about the same thickness, consisting of several rows of sclerenchymatous cells. The cells near the outside are about 15 to 30 μ in diameter, rounded or radially elongated, nearly isodiametric, and provided with very thick pitted walls. Towards the interior of the layer the cells are larger (25 to 60 μ in length), radially elongated, and possess thinner walls.

This sclerenchymatous tissue passes rapidly into the parenchymatous tissue of the pulp, which should be next examined. From this it is separated in older fruits by a few layers of cork cells.

Examination of the Pulp

Take a portion of the dry pulp, cut sections from it, and transfer them to alcohol. Mount in water, and stain if necessary with a dilute aqueous solution of Bismarck brown so as to render the thin transparent cell walls more easily visible. The section exhibits very large, thin-walled, more or less rounded, parenchymatous cells, with large intercellular spaces. The areas of contact are flattened and pitted, often showing in consequence a beaded appearance when cut transversely. The walls are mostly cellulose ; here and there a little lignification can be detected.

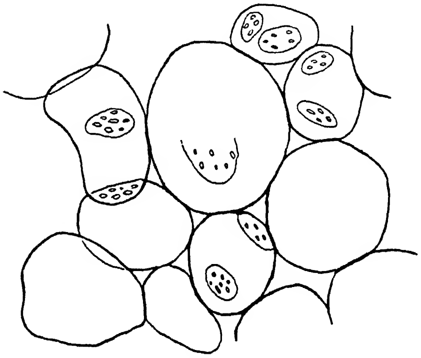

FIG. 144.—Colocynth Fruit, section of the dry pulp. × 65.

This tissue is traversed by fibrovascular bundles containing tubular idioblasts, in which the colocynthin is secreted, but for present purposes these may be neglected.

Examination of the Seed

Preparation and Examination of Sections.—Select some ripe (dark-coloured) seeds, and soak them for several days in water. Split one parallel to the flat surface, and embed one of the halves in cork. Cut transverse sections.

Examine one in glycerin. The epidermis consists of radially elongated cells about 60 μ long and 20 μ wide. The outer tangential walls appear to be very thick, but a close inspection shows that the cell walls are covered by a transparent homogeneous membrane. This, according to Hartwich,[1] is the inner epidermis of the pulp of the fruit, the cells of which collapse and become adherent to the seed. The radial walls of the epidermal cells exhibit in section a number (about ten or twelve) of longitudinal bars of thickened wall which alternate with as many strips of unthickened wall, the bars tapering from base to apex (fig. 145, *ep.*). Hence in surface view these cells appear conspicuously beaded. The contents of the epidermal cells of ripe seeds are brown, and yield the tannin reaction with ferric salts; to them the brown colour of ripe seeds is due. In unripe seeds the epidermal cells are destitute of this brown substance, and in very young seeds they are often collapsed, expanding only when treated with strong solution of potash.

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[1] *Archiv d. Pharmacie*, ccxx. 584.

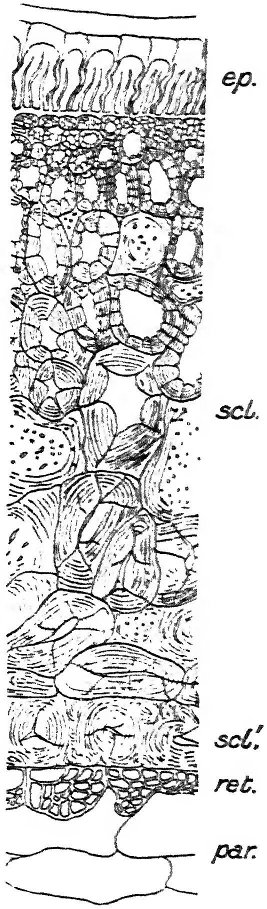

Fig. 145.—Colocynth Fruit, transverse section of part of seed coats. *ep.*, epidermis with outer hyaline layer; *scl.*, sclerenchymatous tissue; *scl.'*, innermost layer of same; ***ret.***, reticulated cells; *par.*, parenchyma. × 240.

Following immediately upon the epidermis is a mass, about 350 μ thick, of sclerenchymatous cells with very thick, yellowish, striated walls (fig. 145, *scl.*). These cells are small (about 10 μ in diameter) near the epidermis, but increase in size towards the interior (50 to 70 μ); the walls also increase in thickness until the inner cells are almost completely filled and the cavity can with difficulty be distinguished. These inner cells do not appear well defined, as they are often irregular in shape. The innermost layer is rather sharply distinguished from the others, except in the acute angles of the seed, where all the cells assume an elongated shape with sinuous or jagged walls (see later, isolation by potassium chlorate and nitric acid).

Next to the sclerenchymatous layer is a single layer of reticulated cells about 90 μ long and 60 μ wide, but varying considerably (fig. 145, *ret.*). They often exhibit dome-shaped projections towards the interior of the seed. The reticulations are lignified. This layer of reticulated cells is followed by a layer of thin-walled parenchyma (fig. 145, *par.*), through which runs the raphe, which is easily seen as a fibrovascular bundle of considerable size near the acute edge of the seed.

Next to the parenchyma is a layer of collapsed cells, in which little structure can be discerned, separated from the embryo by a single layer of distinct flattened cells about 15 to 20 μ long (fig. 146, *4*). These latter are probably the inner epidermis of the endosperm, the remainder of which has collapsed to form the tissue referred to. Although but little structure can be discerned in the transverse section, surface preparations will yield useful information concerning this layer.

Proceed next to isolate and examine the tissues of which the seed coats consist.

Separation of the Component Tissues.—Split some seeds longitudinally, remove the kernels, and digest the seed coats in 5 per cent. solution of potash in a water-bath.

Dissect off the epidermis. The alkali swells the outer thickened walls (or collapsed layer of cells) of the palisade epidermis, but the cells can be isolated and the shape well seen.

Dissect or tease off the inner layers ; four distinct layers of cells can be distinguished :

(i) Long, narrow, thin-walled cells, without intercellular spaces (fig. 146, *2*).

(ii) Large, thin-walled, parenchymatous cells with very small intercellular spaces (fig. 146, *3*) ; the walls of the cells appear beaded in optical section and spirally striated in surface view. They often adhere so closely to the preceding layer as to make it appear that the cells of that layer are striated.

(iii) Rounded cells (fig. 146, *1*) ; these generally adhere to layers *2* and *3*, and are with difficulty visible.

(iv) Delicate, nearly isodiametric cells, each containing a globule of oil (fig. 146, *4*). This tissue is probably the epidermis of the remains of the endosperm.

The treatment with potash, while permitting the separation of the softer tissues, is insufficient to separate the lignified cells of the sclerenchymatous layer. To effect this, macerate the seed coats with potassium chlorate and nitric acid, wash, and tease out. A most varied assortment of sclerenchymatous cells will be found. Some are small, regular, and nearly isodiametric; they are derived from the outer portion of the layer;

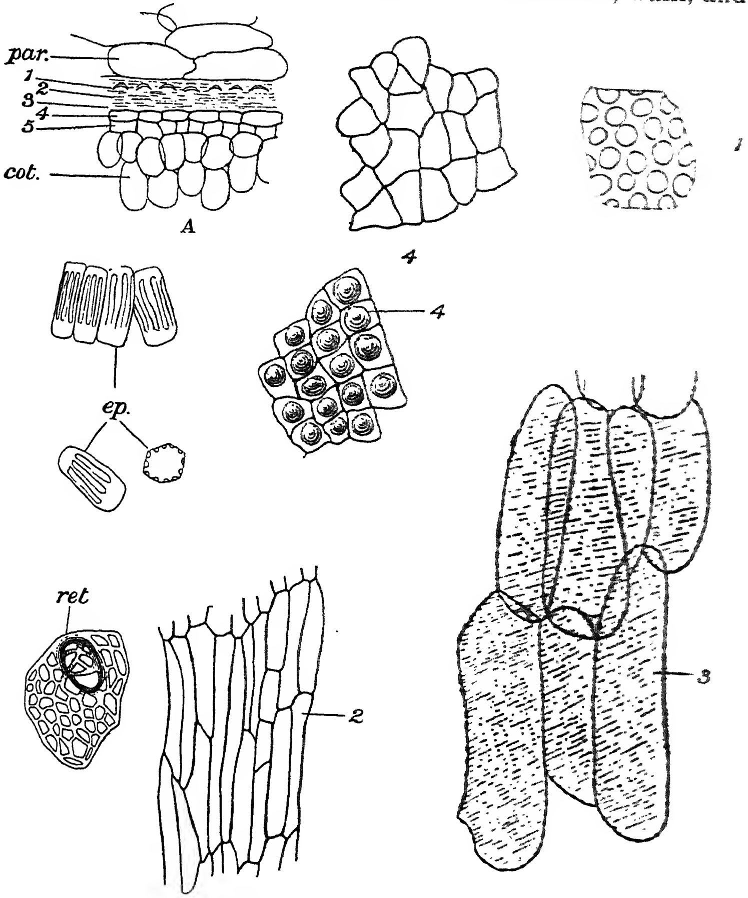

Fig. 146.—Colocynth Fruit, elements of the seed coats. *ep.*, epidermis in section and surface view; *ret.*, reticulated cells within the sclerenchymatous layer, some of the elements of which are shown in figs. 145 and 147; *A*, section of margin of cotyledon, with tissues between this and the reticulated cells; *cot.*, cotyledon; *par.*, parenchyma abutting on the reticulated cells; 1, 2, 3, more or less collapsed layers; 4, inner epidermis of remains of endosperm; 5, epidermis of cotyledon. 1, 2, 3, and 4 are also shown in surface view, the last-named with and without oily contents. × 240.

other larger and less regular cells are derived from the inner layers, while the innermost row of all consists of very thick-walled cells with sinuate outline from which forked projections

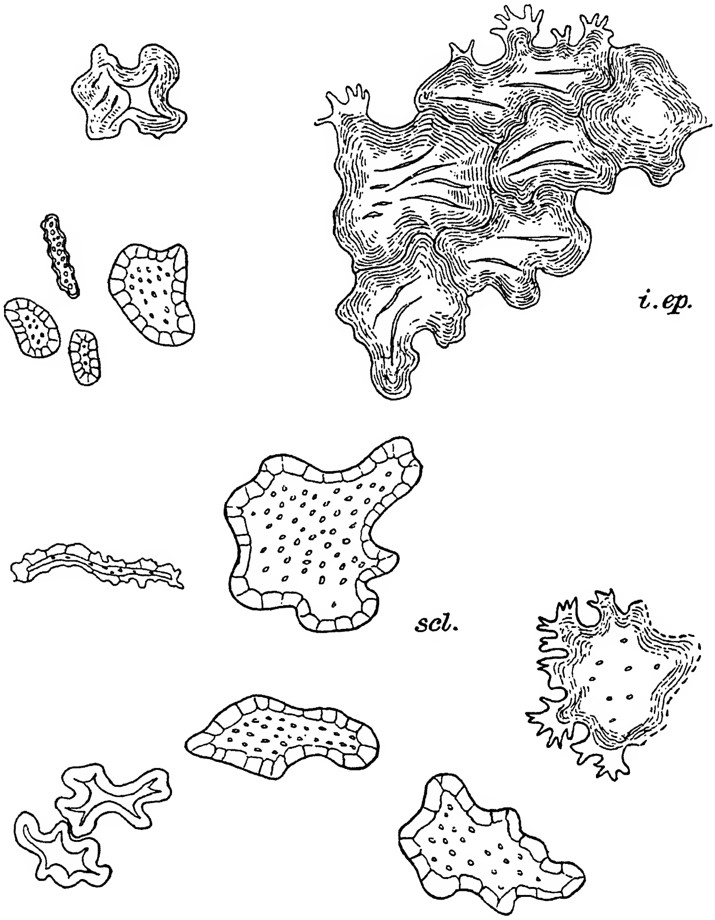

FIG. 147.—Colocynth Fruit, sclerenchymatous cells of seed coat; isolated by potassium chlorate and nitric acid. *i. ep.*, inner epidermis of outer integument. × 240.

stand out, which dovetail with corresponding indentations in the walls of neighbouring cells. There are also long narrow cells with irregular jagged walls; these are derived from near the micropyle, and from the inner layers near the acute edge of the seed.

Next examine the kernel of the seed.

Open a seed and remove the kernel as carefully as possible. Embed it in pith, and cut several transverse sections, which should be transferred to a small corked tube containing ether or ether-alcohol. After maceration for fifteen to thirty minutes pour off the ether and transfer the sections, with the aid of a little alcohol, to a dish.

Mount a thin one in picric acid solution. The cells are filled with very small aleurone grains, each cell containing twenty or more. The grains are ovoid or nearly rounded, and average from 3 to 5 μ in diameter, although they attain as much as 7 μ. Each contains a small rounded body which does not stain with picric acid and resembles a globoid. Irrigate with dilute (0·3 per cent.) solution of potash; the grains dissolve rapidly and entirely, leaving no trace of the globoid body. The cells now exhibit their shape well. On the upper side they are radially elongated and resemble palisade tissue; on the lower they are more nearly isodiametric. They are covered by an epidermis composed of small delicate thin-walled cells.

Mount a section (not previously defatted) in chloral hydrate; oil globules exude in abundance.

Mount a defatted section in iodine water or dilute iodo-potassium iodide; there is no starch present, at least in ripe seeds.

No calcium oxalate can be found, either in the seed coats or in the kernel.

Most of the seeds that are present in the commercial drug are unripe; the student should, therefore, also cut sections of unripe seeds, and compare them with the ripe. The following differences may be observed:

The epidermis of the seed coat is less developed; the bar-like thickenings have often not formed; sometimes even the epidermal cells are indistinct; the sclerenchymatous cells are much less thickened. A study of the seed coats of unripe seeds contributes materially to the proper understanding of that of the ripe seeds.

Diagnostic Characters.—The following cells and cell contents are the most important from a diagnostic point of view:

1. The large, thin-walled cells of the pulp; powdered colocynth pulp should consist principally of fragments of these; they stain blue with chlorzinciodine.

2. The epidermis of the pericarp; this, if present to any appreciable extent in powdered colocynth, would indicate the use of unpeeled or badly peeled fruits.

3. The palisade epidermis of the seed coat, with its characteristic thickenings; the fact that these thickenings do not branch towards the apex of the cell distinguishes this seed from certain other cucurbitaceous seeds.

4. The sclerenchyma of the seed coat, especially the cells of the innermost layer.

5. The spirally striated cells; most cucurbitaceous seeds contain these.

Additional means of determining the presence of seed in the powdered drug may be found in

6. The aleurone grains.

7. The presence of oil.

Neither pericarp nor seed contains either starch or calcium oxalate.

Examination of the Powder

Proceed next to the examination of the powdered fruit, taking care to obtain powdered *fruit* and *not pulp*.

Moisten a little with alcohol, add water, and examine. Observe in this preparation abundant *débris* of parenchymatous tissue evidently consisting of very large cells. Sometimes these fragments exhibit pitted areas or transverse walls, but such are not very readily seen. There is also a quantity of granular matter present and yellowish masses which do not readily show their structure; they are fragments of sclerenchymatous tissue, but they are better examined in another medium. Allow chlorzinciodine to flow on; the colourless fragments of parenchyma turn bluish violet.

Moisten another portion with alcohol, allow this to become nearly dry, and add a very small drop of picric acid solution; mix well, and after a minute or two add a drop of glycerin; mix, and examine. The small oval aleurone grains can be easily found; they are present in numbers, and are readily detected by their yellow colour. The small rounded globoid (?) appears faintly orange-red in colour. Carefully examine this slide for fragments of the cotyledons. These may be detected by the now yellow aleurone grains with which the cells are packed; they are not, however, very readily observed.

Warm another portion in chloral hydrate. The sclerenchymatous tissue is conspicuous; it generally has a yellow colour. It is present as isolated cells, or groups of cells, or as transverse fragments of the seed coats extending from the epidermis to the innermost sclerenchymatous layer. Observe such cells or fragments of sclerenchymatous tissue as have much thinner

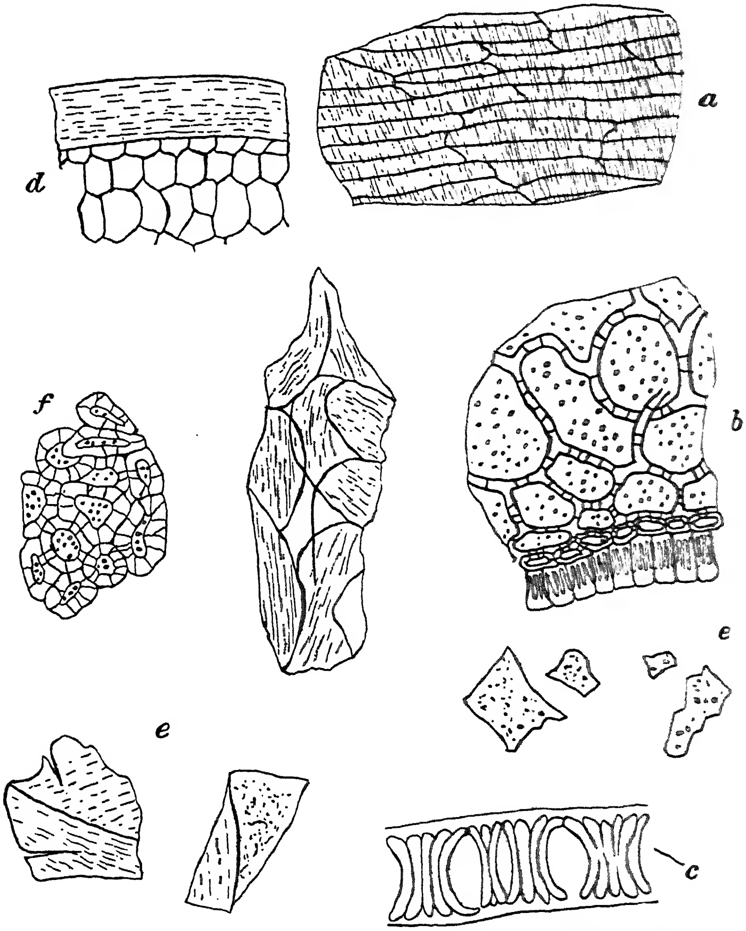

FIG. 148.—From Powdered Colocynth Fruit. *a*, striated cells of inner seed coat (compare 2 and 3, fig. 146); *b*, epidermis and subjacent sclerenchyma of unripe seed; *c*, inner epidermis of outer integument of unripe seed; *d*, fragment of cotyledon with inner layers of seed coat; *e*, fragments of cells of pulp; *f*, sclerenchyma of nearly ripe seed. × 240.

walls and especially differ in the innermost layer; they are derived from unripe seed, and are commonly more numerous than those derived from ripe seed. These fragments are not very readily distinguished from the sclerenchyma of the rind, but the cells of the latter usually exhibit distinct radial elongation and are more angular and comparatively thin-walled. Some of the sclerenchymatous cells are long and narrow, with jagged edges; these come from the acute edge of the seed.

The delicate striated cells from the collapsed layers are very readily found; they generally occur in fragments of considerable size exhibiting their surfaces (fig. 148, *a*).

The epidermis of the seed coat may also be found; the cells are often in profile, but sometimes in surface view; although the outer wall is more or less swollen, the bar-thickenings are always conspicuous and are very characteristic.

The epidermis of the fruit may sometimes be detected; it is easily recognised, especially when exhibiting its transverse section, but should be present only in very small quantity.

Stain another portion with phloroglucin and hydrochloric acid; the sclerenchymatous cells are coloured deep red.

Examine next the powdered pulp of commerce. Here, too, fragments of the tissue of the seed may be found, but they should not be so numerous as to raise suspicion of adulteration.

Mount a little in iodine water or chloral iodine to prove the absence of any but occasional, very minute, isolated starch grains (derived from very young seeds).

Capsicum Fruit (Chillies)

Source.—The fruit of *Capsicum minimum*, Roxb.

Preparation.—Capsicum fruit should be divided, for complete examination, into the following parts, each of which should be examined separately:

(*a*) Pericarp; (*d*) Calyx;
(*b*) Dissepiment; (*e*) Stalk.
(*c*) Seed;

Examination

(*a*) **Pericarp.**—Select a few good Zanzibar or Sierra Leone chillies, and soften them by exposing them to a moist atmosphere for twelve hours. Carefully separate the pericarp, cut into suitable strips, embed in pith, and cut transverse sections. Examine in chloral hydrate.

Observe the outer epidermis. The outer tangential walls are much thickened, and so also are the radial walls, but only for about two-thirds of their length, the remaining inner third being thin. Below the epidermis is parenchymatous tissue containing much reddish-coloured oil, and here and there a small bicollateral bundle and cell filled with sandy calcium oxalate. Between this parenchyma and the inner epidermis there is a row of very large cells, so large that they might be

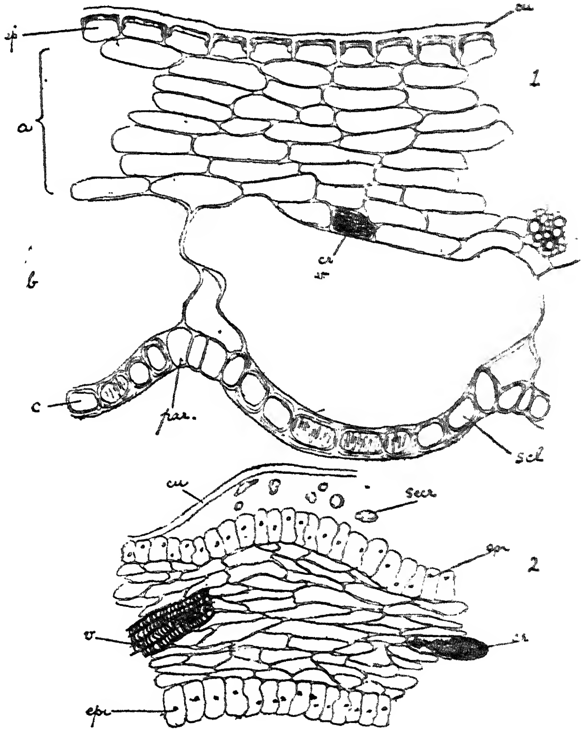

FIG. 149.—Capsicum Fruit. 1, transverse section of pericarp; *a*, parenchyma; *b*, large-celled layer of same; *c*, inner epidermis; *cr.*, sandy crystals of calcium oxalate; *par.*, parenchymatous cells, and *scl.*, sclerenchymatous cells of inner epidermis. 2, transverse section of dissepiment; *cr.*, sandy crystals of calcium oxalate; *cu.*, cuticle, raised by the secretion, *secr.*; *epi.*, epidermis; *v.*, vascular bundle. × 170. (Wallis.)

mistaken for spaces. The inner epidermis itself is composed of two kinds of cells—viz. sclerenchymatous cells, with thick, pitted, lignified walls, and thin-walled parenchymatous cells. The latter occur over the radial walls of the large subepidermal cells, and hence in surface sections exhibit a reticulate distribution corresponding to the walls of the cells over which they are situated; the sclerenchymatous cells occupy the intervening spaces, and the groups of these cells correspond, therefore, to the cavities of the large cells.

From another fruit, softened as described, separate the pericarp; observe with a lens the bladdery appearance of the inner surface, due to the large cells just alluded to. With dissecting needles and forceps strip the epidermis from the inner surface and examine in chloral hydrate, taking care that the cuticle is uppermost. If observation is impeded by the oil, which is often present in considerable quantity, remove this by maceration in ether-alcohol. The sclerenchymatous cells can now easily be seen in surface view; they are approximately isodiametric, with thickened, pitted, lignified, sinuous walls, and are arranged in elongated oval groups between which there are narrow strands of delicate parenchymatous cells. The latter are not easily made out, and require careful adjustment of the light and focus.

Warm a fragment of the pericarp in chloral hydrate; the upper epidermis shows well; the cells are mostly four-sided, and have a delicately striated cuticle; they are often arranged in rows of seven or more, which appear to have been formed by the transverse division of one large elongated cell—a peculiarity that is characteristic of this species of *Capsicum*.

(*b*) **Dissepiment.**—Cut open another fruit, and carefully remove the thin membranous dissepiment. Examine the surface in chloral hydrate after warming. The epidermis is formed of thin-walled polygonal cells, from which the cuticle is in great part separated, forming an indistinct, structureless, crumpled membrane over the epidermal cells. In the parenchymatous tissue cells filled with sandy crystals are to be seen.

A transverse section through the delicate dissepiment shows that the cuticle has in places been raised from the epidermis by the secretion of oily drops from the epidermal cells; these are said to contain the capsaicin to which the fruit owes its pungency.

(*c*) **Seed.**—Soak a few seeds in water until sufficiently soft to cut. Embed one in pith, and cut transverse sections with a sharp razor; if it is not held sufficiently firmly by the pith, fix it between the two halves of a velvety cork.

Examine the sections in chloral hydrate. Observe the epidermis carefully. It is composed of cells that exhibit a very remarkable thickening. The inner tangential wall is moderately thickened in the centre, more strongly in the angles, but on the radial walls the thickening gradually diminishes until, near the outer tangential wall, it is very slight. The section therefore exhibits an irregular horseshoe thickening.

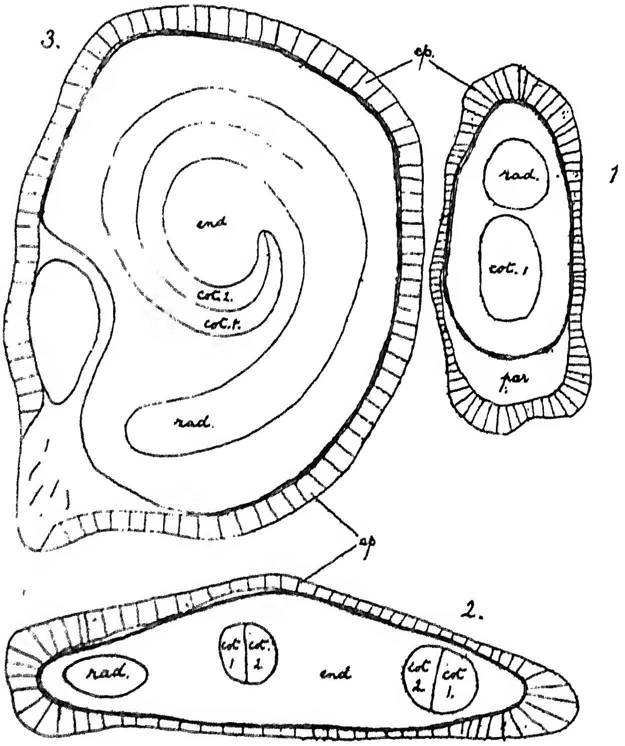

FIG. 150.—Capsicum Fruit, sections of the seed. 1, transverse; 2, longitudinal, cut at right angles to the flat surfaces; 3, longitudinal, parallel to the flat surfaces. (Wallis.)

Stain a section with chlorziniodine; on the outside there is a delicate cuticle; next to this a layer of cellulose, and within the layer of cellulose a lignified layer; the general cavity of the cell is also lined with a layer of cellulose.

The cells on the edge of the seed are larger and more strongly radially elongated than those on the flat sides.

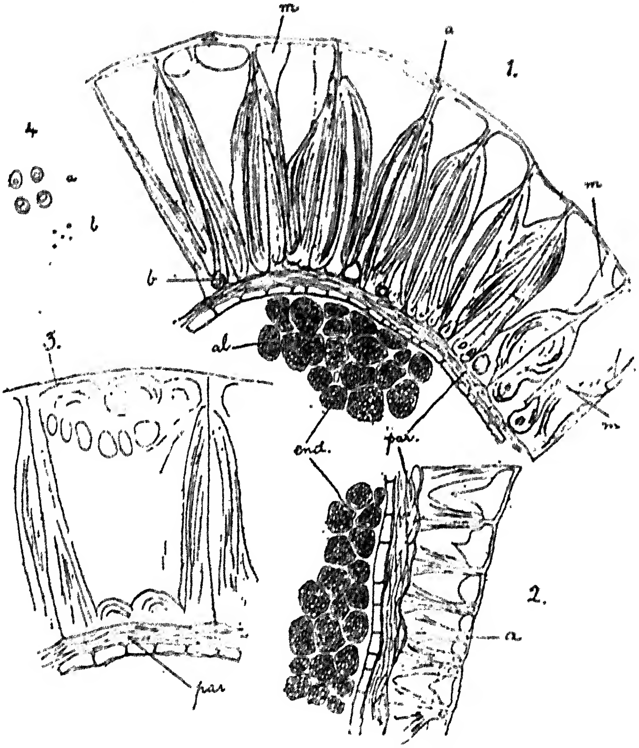

FIG. 151.—Capsicum Fruit. 1, transverse section of seed coat and portion of endosperm, at edge of seed; 2, the same from flat surface; 3, seed coat at edge of seed, cut parallel to the flat surface; *a*, processes from the upper surfaces of epidermal cells; *b*, processes from the lower surfaces of the same; *al.*, aleurone grains; *end.*, endosperm; *m*, delicate cellulose membrane in epidermal cells; *par.*, collapsed parenchyma of seed coat. All × 150. 4, aleurone grains, more highly magnified; *a*, before, *b*, after treatment with potash. (Wallis.)

Fix a seed by its flat surface on a cork, using a little mucilage; allow it to dry; cut surface sections, and examine in chloral hydrate. The cells appear thickened, and the outline wavy.

Split a few seeds parallel to the flat surface and also transversely; remove the endosperm; macerate the fragments with potassium chlorate and nitric acid ; wash ; tease out and examine the separated cells. They have the same appearance as they showed in surface section, but they exhibit, in addition, little irregular projections which dovetail with those of neighbouring cells.

Examine the kernel as directed for colocynth seeds ; it consists of thin-walled cells, in which aleurone grains and oil are the principal reserve materials.

Calyx and Stalk.—Examine these by the methods previously detailed for leaves and stems.

The upper epidermis of the calyx is characterised by numerous multicellular glandular hairs containing a yellowish secretion ; stomata occur on the under surface only, and each is surrounded by three or four cells, of which one is smaller than the others. The mesophyll contains cells filled with sandy crystals of calcium oxalate.

The stalk has an epidermis consisting of elongated cells and bearing an occasional glandular hair ; the pericycle contains well-developed fibres.

Powder

Powdered chillies may be examined as follows :

(1) In water or dilute glycerin. Note the abundance of red globules of oil. Fragments of the epidermis of the seed and pericarp may be observed, but are better examined in the following preparation. Aleurone grains are not easy to identify.

(2) Warm a little in chloral hydrate, cool, and examine. In this preparation fragments of the epidermis of the seed coat, with its remarkable sclerenchymatous cells of yellowish colour, are usually very conspicuous. Sometimes they exhibit their surface, especially if the fragments are large, but smaller ones or isolated cells often present their section. The inner epidermis of the pericarp, with its thick-walled lignified cells, is also easily found. Sometimes these cells are attached to the non-lignified parenchyma, with which they alternate, but more often they are separated. The striated outer epidermis is also to be found, but not quite so easily. Portions of the endosperm, the cells of which have rather thick walls, are not difficult to detect, while fibres and vessels from the stalk, calyx, &c., are scattered in every preparation.

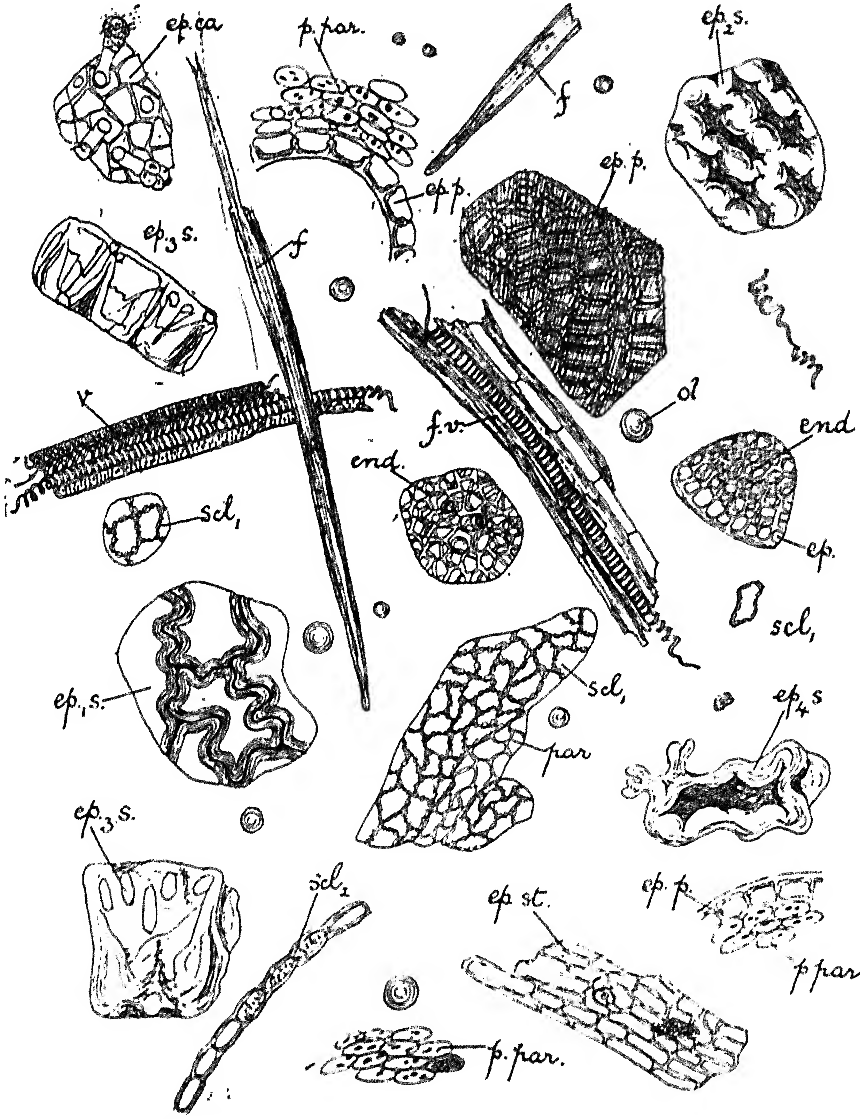

FIG. 152.—Capsicum Fruit. Powder. *end.*, endosperm; *ep.*, epidermis of same; *ep. ca.*, upper epidermis of calyx; *ep. p.*, outer epidermis of pericarp; $ep_1s.$, epidermis from flat surface of seed; $ep_2s.$, epidermis from edge of seed; $ep_3s.$, epidermis of seed, side view; $ep_1s.$, isolated epidermal cell of seed coat; *ep. st.*, epidermis of stalk; *f*, sclerenchymatous fibres; *f.v.*, fibro-vascular bundle; *ol.*, oil; *par.*, parenchyma of inner epidermis of pericarp; *p. par.*, parenchyma of pericarp; scl_1, sclerenchyma of inner epidermis of pericarp, seen from above; scl_2, the same, side view. × 108. (Wallis.)

(3) Defat a little of the powder with ether-alcohol, dry, and mount in chloral hydrate. Compare this preparation with No. 2.

(4) Decolourise a little of the defatted powder, wash, and treat as follows :

(*a*) With Soudan red : the portions of epidermis bearing a cuticle stain red ;

(*b*) With phloroglucin and hydrochloric acid ; lignified cell walls are stained.

Fruits of other Species of Capsicum.—Wallis[1] has also examined the fruits of *C. annuum* as well as those known in commerce as Japanese chillies, the botanical origin of which has not been definitely determined. The following table shows the chief microscopical features by which these fruits may be distinguished from one another, either in the entire or powdered state.

	C. MINIMUM	C. ANNUUM	JAPANESE CHILLIES
Epidermis	Thick and straight-walled rectangular cells with few pits; often arranged in groups of five to seven in a row and with a uniformly striated cuticle. Size of cells, 25 μ to 60 μ in either direction.	Irregular polygonal cells with evenly thickened walls, traversed by numerous, well-marked, simple pits. The cuticle shows striated ridges. Size of cells, 60 μ to 100 μ long, and 25 μ to 50 μ wide.	Cells with strongly thickened walls and a radiate lumen. The pits only rarely penetrate the whole thickness of the wall. No visible striation. Size of cells, 30 μ to 80 μ long and 15 μ to 45 μ wide.
Hypoderma	Delicate cells with thin cellulose walls.	Several layers of cuticularised collenchymatous cells, having a rounded outline and very few pits.	A single layer of regular polygonal cells with cuticularised fairly thick walls, traversed by numerous pits, which give them a beaded appearance.

Black Pepper

Source.—The unripe fruit of *Piper nigrum*, Linn.

Preparation and Examination of Sections.—Soak a number of peppercorns in water for about twelve hours, when they will be sufficiently softened for examination.

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[1] *Pharmaceutical Journal*, vol. 69, p. 3.

Examine with a lens and find the scar that indicates the point of attachment to the stem; at the apex of the fruit the remains of the stigmas can generally be discerned. Cut the fruit transversely, and then cut each half again at right angles to the transverse surface. Embed one of these quarters in pith, so that the half of the transverse section is presented for cutting. When cutting the sections, take care that the edge of the razor cuts the pericarp first (this should therefore be towards the operator's left and the perisperm towards his right).

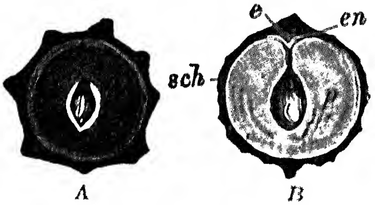


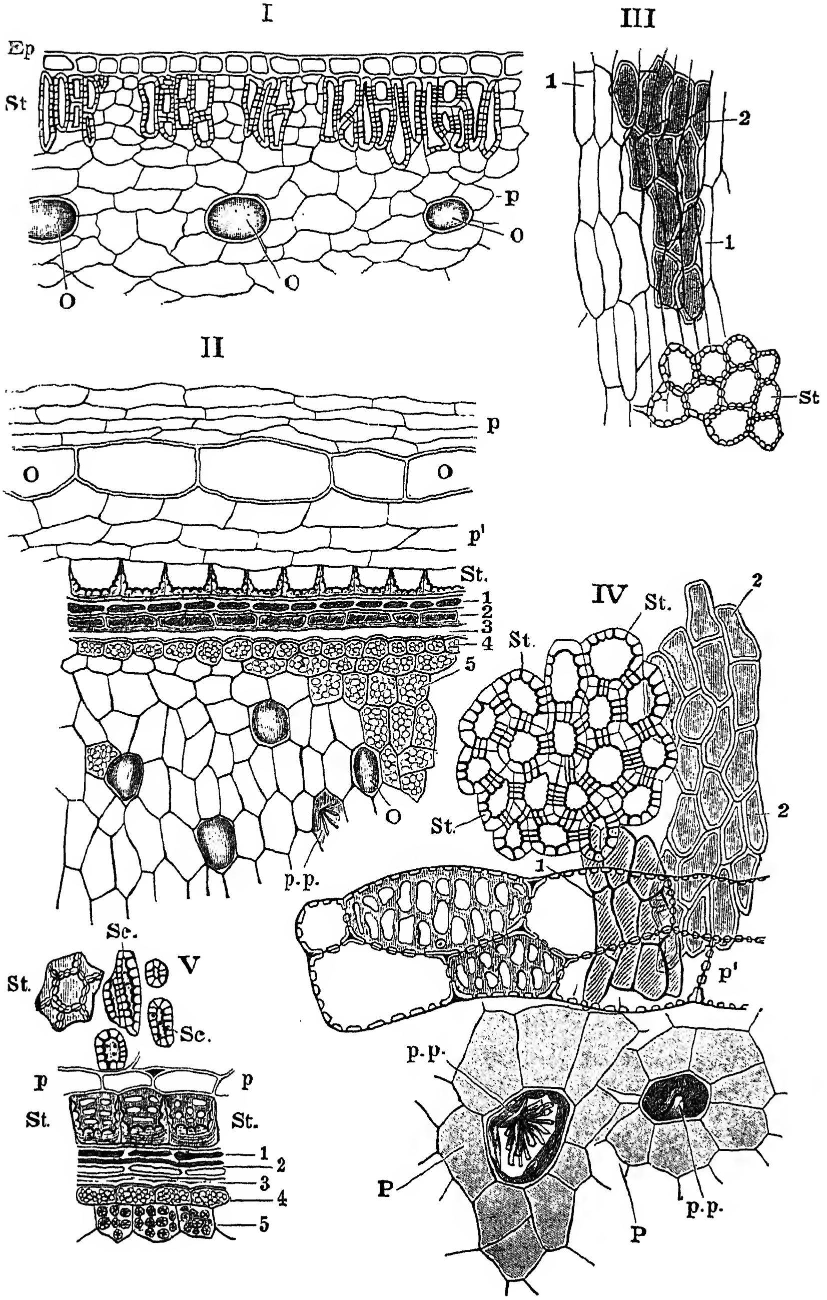
Fig. 153.—Black Pepper. *A*, transverse section; *B*, vertical section, showing pericarp, *sch*; perisperm, *p*; endosperm, *en*; and embryo, *e*. Magnified. (Tschirch.)

Examine a section in water under the low power; the brownish tissue of the pericarp is well differentiated from the whitish perisperm, the cells of which are polygonal and packed with minute starch grains.

Mount a thin section in solution of potash, and warm gently till the starch is gelatinised; examine first under the low, then under the high power.

The epidermis is formed of small cells covered with a thick cuticle; they are often best seen near the thin ends of the section; they contain a brownish granular substance in which minute prismatic crystals of calcium oxalate are embedded; six or eight such crystals can often be seen in each cell (especially in surface preparations).

Fig. 154.—Black Pepper. I, transverse section of the outer portion of the pericarp; *Ep.*, epidermis; *St.*, sclerenchymatous cells of the hypodermal (or subhypodermal) layer; *p*, parenchymatous cells; *O*, oil cells. II, transverse section of the inner portion of the pericarp and part of the perisperm of the seed; *p*, parenchyma; *O*, oil cells; *p'*, subjacent parenchyma; *St.*, inner layer of sclerenchymatous cells; 1, 2, 3, layers of the seed coats; 4, epidermis of perisperm; 5, perisperm with oil cells, *O*, in one of which crystals of piperine, *p.p.*, can be seen. III, sclerenchymatous cells of the inner layer, together with the subjacent seed coats; 1 and 2, surface view. IV, surface view of some of the tissues; *St.*, sclerenchymatous cells of inner layer; 1 and 2, subjacent seed coats; *p'*, the reticulated parenchymatous layer exterior to *St.*; *P*, parenchyma of the perisperm, with starch and oil cells, the latter containing crystals of piperine. V, transverse section of the inner layers of the pericarp, lettering as in II. (Vogl.)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Below the epidermis, sometimes immediately abutting upon it, sometimes separated from it by a row of small parenchymatous cells, is a layer of sclerenchymatous cells. This layer is not continuous, but interrupted at intervals by small groups of parenchyma. The sclerenchymatous cells themselves vary considerably, both in size and in shape. Some are isodiametric, 15 to 20 μ in diameter, and square or rounded in outline; but the majority are radially elongated, and frequently attain 100 μ in length and 20 μ in width. They often exhibit brown contents. These cells are characteristic of pepper, and should therefore be carefully examined. The parenchymatous tissue of this region often contains brown granular matter in which numerous small crystals of calcium oxalate can be detected. The latter are especially visible when a section cleared by potash or chloral hydrate is examined with polarised light under the high power.

Following upon this outer sclerenchymatous zone is a parenchymatous tissue which constitutes the bulk of the pericarp and is rather sharply differentiated into an outer and inner portion separated by a dark, often greyish or brownish line, which, on careful examination, is seen to consist simply of compressed thin-walled cells. This region of the pericarp is traversed by fibrovascular bundles, which, in transverse section, exhibit a few small spiral vessels and often sclerenchymatous fibres, the nature of which can be determined later from a potash maceration preparation.

The outer portion of this parenchyma is composed of about twelve rows of tangentially elongated parenchymatous cells, which contain, as may be determined by examining a section in iodine water, numerous minute scattered starch grains. Here and there in this tissue an oil cell may be found containing a globule of oil; such globules are most evident in moderately thick sections after treatment with potash.

The inner portion of the parenchyma is especially characterised by the presence of a large number of oil cells, which are arranged in an almost continuous ring. The innermost row or two of the cells of this tissue are often reticulately thickened (especially in riper fruits); they abut directly upon a single row of cells that are conspicuous by reason of their bright colourless walls. These conspicuous cells exhibit a very remarkable horseshoe thickening on the radial and inner tangential walls, coarse pits being visible near the points of the horseshoe. This layer is the inner epidermis of the pericarp, and is of great importance, as it is one of the most characteristic layers of the pepper fruit.

Between the horseshoe cells and the perisperm is a narrow brown ring, which, in favourable sections and under a high power, can be resolved into three distinct layers—viz. an outer pale brown, a middle dark brown, and an inner colourless one. As a rule, these layers do not exhibit more than indications of cellular structure, but such indications become more distinct after treatment with Schulze's maceration mixture; these layers, which constitute all that remains of the seed coats, are, however, best examined in surface sections or potash preparations.

Examine a section in water. The perisperm is principally composed of large thin-walled polygonal cells packed full of minute starch grains. Among these cells numerous others may be distinguished by their yellowish oily contents. These are oil cells. They are characterised by the blood-red colour the contents assume with concentrated sulphuric acid, a reaction due to the piperine they contain, which may occasionally be found in prismatic crystals embedded in the oleo-resin.

The perisperm cells that abut on the seed coats are smaller and contain aleurone grains.

The starch occurs in minute simple grains or larger rounded or ovoid compound grains, the latter consisting of a large number of minute component grains. To examine the starch, slightly crush a section and observe the minute grains. Allow chloral hydrate to flow on to a very thin section; large oval or rounded compound grains can then easily be seen, embedded in a mass of simple grains. After the starch has been gelatinised, many of the cells will be seen to contain a prismatic crystal of calcium oxalate as well as a small body of irregular form and unknown nature.

Examination of Surface Sections.—Select a smooth (nearly ripe) fruit, embed it in pith so that the surface of the fruit is just below that of the pith, and cut a series of surface sections. Transfer them all to a slide, keeping the upper surface uppermost, clear, and examine.

The first section should exhibit the epidermis in surface view; the cells, which are often best seen near the edge of the section, are small and rounded-polygonal in shape, and contain numbers of minute crystals of calcium oxalate.

The second section should exhibit the distribution of the outer sclerenchymatous cells; they occur in patches, separated from one another by brownish parenchymatous cells, which, like the epidermal cells, contain calcium oxalate.

The following sections should show, near the middle, the outer parenchymatous tissue, the inner parenchymatous tissue, the horseshoe layer, the seed coats, and the perisperm successively.

The horseshoe layer should be particularly examined, as it always presents its surface view in powdered pepper. The appearance of the cells varies a little with the point at which they are focussed; with high focus they appear rather thin-walled and pitted; as the focus is lowered, the walls appear thicker and the pits disappear.

Of the layers of the seed coats the middle dark layer is the most important; it may be recognised by its dark reddish-brown colour, the cells being elongated-polygonal in shape. These layers of the seed coats may be further examined in a potash maceration preparation.

Examination by Maceration in Potash.—Digest a few peppercorns in solution of potash in a water-bath; strip the pericarp and seed coats from one of them, and disintegrate as thoroughly as possible with the needles. Examine, and identify the various tissues and elements by comparison with the sections.

Examination of Powdered Pepper.

(1) Mount a little in water or dilute glycerin. The bulk of the powder consists of angular colourless fragments of perisperm, which present a granular appearance, due to the minute starch grains with which the cells

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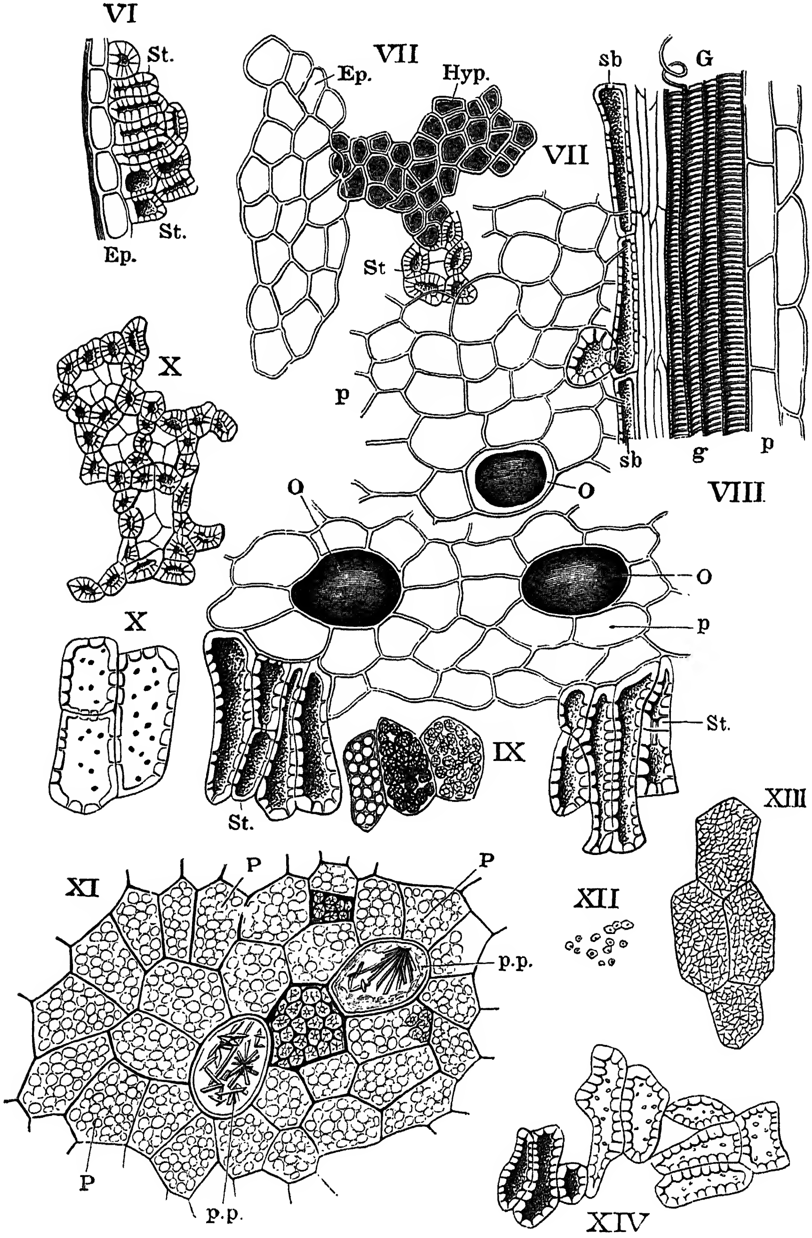
Fig. 155.—Black Pepper (*continued*). VI, epidermis, *Ep.*, with subjacent sclerenchymatous cells, *St.* VII, *Ep.*, epidermis; *Hyp.*, hypoderma; *St.*, hypodermal sclerenchyma, all in surface view; *p*, parenchyma of pericarp, with oil cells, *O*, and hypodermal sclerenchymatous cells, *St.* VIII, fibrovascular bundle in radial section; *g*, vessels; *p*, parenchyma; *sb*, sclerenchymatous cells. IX, perisperm cells with starch, on the left a cell in which the starch has been gelatinised. X, upper figure, hypodermal sclerenchyma, surface view; lower figure, sclerenchymatous cells from the mesocarp. XI, portion of the perisperm. XII, isolated starch grains. XIII, cells of the perisperm, with starch. XIV, sclerenchymatous cells. (Vogl.)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X
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St.
IX
St.
XIII
XI
P
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p.p.
P
p.p.
XII
XIV

are packed. In addition to these fragments, there are to be found the cell contents of some of the cells, which have fallen out intact, and also numerous starch grains, which are either isolated or in groups of a few; these grains can be identified as pepper starch; irrigate with iodine water in order to prove their nature.

In this preparation fragments of the reddish-brown seed coats and other *débris* can be seen, but they are better examined after removal of the starch.

(2) Moisten a little with a small drop of alcohol, allow it to stand a few moments, and add a small drop of dilute glycerin. The preparation will become cloudy, owing to the separation of volatile oil. Cover, and after five minutes examine again. Abundance of long narrow prismatic crystals will be found, either isolated or arranged in radiating groups; these are crystals of piperine.

(3) Boil 1 gramme for ten minutes with 20 c.c. of water to which 2 c.c. of hydrochloric acid (sp. gr. 1·16) have been added; allow the cell *débris* to deposit, wash once with dilute (about 1 per cent.) solution of potash, and finally with water. Mount a little in chloral hydrate, and examine. Observe the following characteristic portions of the pepper fruit.

(a) *Fragments of the parenchymatous tissue of the perisperm.* The cells are large, elongated, and often angular, and have very thin walls without visible pits. Here and there they appear to possess dull granular contents (remains of the starch), and occasionally a rounded cell with refractive walls may be found (oil cell), in which sometimes a globule of oil may be seen.

(b) *Similar fragments from the pericarp*; in these the parenchymatous cells are smaller, the oil cells are also smaller; the fragments often have a pale brownish colour, and contain the small fibrovascular bundles.

(c) *Fragments of the seed coats.* These are more conspicuous in white pepper, which is prepared from riper fruits than black pepper. They have a bright reddish-brown colour, and are easily seen. Discernible in these fragments are usually ·

α. The hyaline layer, colourless, with very distinct cell walls.

β. The inner seed coat, dark reddish-brown; cells often less distinct.

γ. The outer seed coat, yellowish-brown, visible here and there.

(d) *Fragments of the inner sclerenchymatous layer.* They are usually brownish in colour from the seed coats which adhere to their lower (inner) surface; they often exhibit, especially near the edges of the fragments, the large reticulated parenchymatous cells that abut upon their upper (outer) surface. The small polygonal cells of which this sclerenchymatous layer consists and their pitted walls render it easy to identify.

(e) *Fragments of the outer sclerenchymatous layer.* These are absent or very rare in white pepper, but are numerous in black. They are conspicuous by reason of their dull, dark, brownish or yellowish colour (not bright reddish-brown). They usually exhibit their surface view, and are then sharply characterised by the small sclerenchymatous cells which present somewhat varying, often irregularly polygonal, sections, and are arranged in small groups separated by dark-coloured parenchyma. The epidermis often adheres to them, but is not readily seen.

(f) *Isolated sclerenchymatous cells*, or small groups of such. These are derived chiefly from the outer sclerenchymatous layer. Long narrow cells may occasionally be found; these are the sclerenchymatous cells that accompany the bundles in the pericarp.

Fennel Fruit

Source.—The dried ripe fruit of *Fœniculum capillaceum*, Gilib.

Preparation and Examination of Sections.—Select if possible some Saxon or other bold fennel fruits, and soak them in dilute (50 per cent.) glycerin for twenty-four hours or longer. Split a soaked fruit into its two mericarps, embed one in pith, cut a number of transverse sections through the centre and transfer them to alcohol. Mount one in dilute glycerin and examine under the low power (fig. 156). The centre is occupied by the large greyish endosperm, which is surrounded by the pericarp and seed coats, the latter reduced to inconspicuous membranes. In the pericarp the oval vittæ are conspicuous and in each of the ridges there is a large bundle of fibres; a small fibro-vascular bundle accompanied by fibres, situated on the commissural surface, is the raphe. The inner layers of the mesocarp, especially the cells surrounding the vittæ, are often dark brown in colour.

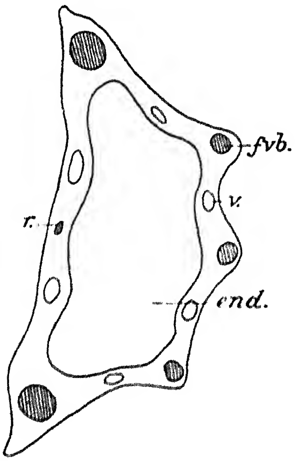

Fig. 156.—Fennel Fruit. Diagrammatic section. *end.*, endosperm; *fvb.*, fibro-vascular bundle; *r.*, raphe; *v.*, vittæ. × 50.

Examine with the high power. If the cells of the epidermis are not distinct, as is often the case, mount a thin section in 25 to 50 per cent. solution of potash. The epidermal cells are small, tangentially elongated or nearly square, with moderately thick walls and a granular cuticle.

Within the epidermis is the mesocarp, bounded on its inner margin by a row of strongly tangentially elongated cells with thin walls of a pale-brownish colour (fig. 157, II); this row of cells, the inner epidermis of the pericarp, exhibits here and there a group of closely approximated, radial walls (chloral hydrate or potash preparation).

Next to the inner epidermis of the pericarp is the outer epidermis of the seed coats, the cells of which are brownish in colour and tangentially elongated and exhibit granular contents (fig. 157, II); examined in potash, they often exhibit oil globules. The remaining cells of the seed coats have collapsed to a hyaline layer, which, however, widens out in the commissural region to a thin-walled parenchyma in which the raphe is embedded.

Examine the mesocarp. In the neighbourhood of the vittæ the cells are thin-walled and tangentially elongated, but, as the ridges are approached, the walls become characteristically thickened and lignified and then exhibit large oval or rounded pits or irregular reticulations. The number of cells thus thickened varies considerably in different fruits. The bundles themselves consist of small thickened and lignified elements, on each side of which is a group of sieve tissue accompanied by a few small tracheids.

In many fruits, especially when ripe, the cells of the mesocarp near the vittæ are brown or even very dark brown in colour and the small intercellular spaces are filled with a brown deposit, probably the result of infiltration and oxidation of the volatile oil. Between the vittæ and the inner epidermis the cells are often strongly tangentially elongated.

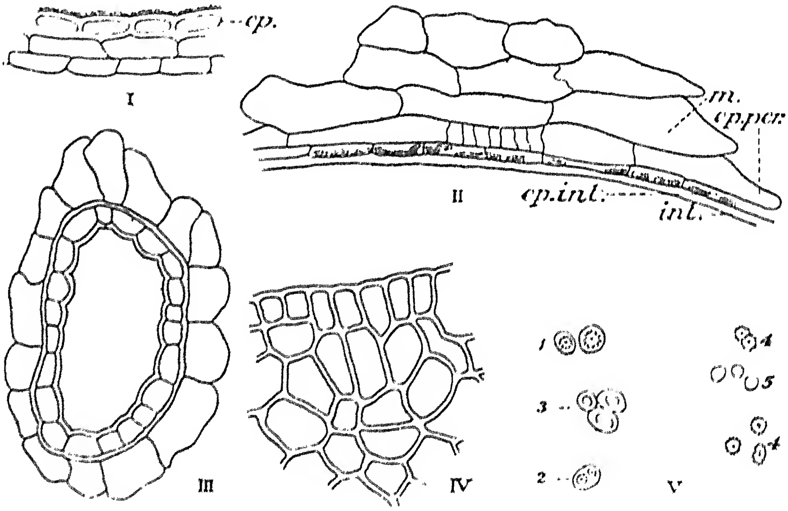

FIG. 157. Fennel Fruit. I, transverse section of outer portion of pericarp; *ep.*, epidermis. × 210. II, transverse section of integument and of inner portion of pericarp; *ep. int.*, epidermis of integument; *ep. per.*, epidermis of pericarp; *int.*, integument; *m.*, mesocarp. × 240. III, transverse sect. of vitta. × 50. IV, transverse sect. of portion of endosperm. × 250. V, aleurone grains, 1 and 2 with rosette of calcium oxalate; 3 with globoids; 4, 4, rosettes; 5, globoids, after solution of ground substance. × 480.

The secreting cells of the vittæ are occasionally visible, but usually they form a collapsed brown layer without distinct structure.

The cells of the endosperm have moderately thick walls and contain aleurone grains, fixed oil and, frequently, an easily visible nucleus.

Examine the aleurone grains as directed on p. 192. They are very small (4 to 10 μ), rounded or oval grains and contain one or two minute (2 to 5 μ) globoids or rosettes of calcium oxalate, the latter with a distinct central point.

Maceration Preparation of Pericarp.—Digest a few ripe fruits with 3 per cent. solution of potash in a water-bath for ten to fifteen minutes, wash with distilled water. Strip off the pericarp from between the ridges and from the commissural surface; separate also the ridges and disintegrate each portion, as far as possible, separately. In the first, look for fragments of the epidermis with an occasional stoma (fig. 158, *ep.*); the colourless cell walls show no intercellular spaces. Fragments of the mesocarp (fig. 158, *m.*) with rounded, thin-walled, colourless or dark-brown empty cells with intercellular spaces occur. Portions of or even entire vittæ (fig. 159, *v.*) are readily recognised by their dark-brown colour; they often exhibit brown transverse dissepiments and show the outlines of small polygonal cells on their walls. To these the inner epidermis of the pericarp (fig. 159, *i. ep.*) often adheres; the cells are elongated, narrow, and often bear evidence of having been produced by subdivision of mother-cells into a number of smaller parallel cells, though these are often oblique to those of neighbouring cells (fig. 159, *i. ep.*).

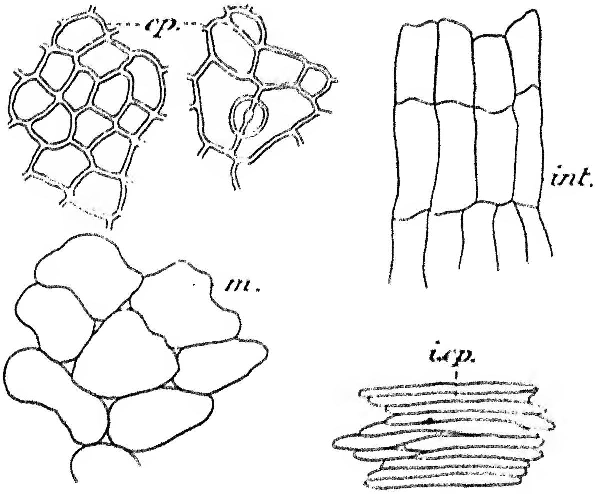

FIG. 158. Fennel Fruit, tissues separated by digestion with solution of potassium hydroxide. *ep.*, outer epidermis of pericarp; *i. ep.*, inner epidermis of pericarp; *int.*, epidermis of integument; *m.*, parenchyma of mesocarp. All in surface view. × 240.

The characteristic spiral, porous or reticulated cells are readily

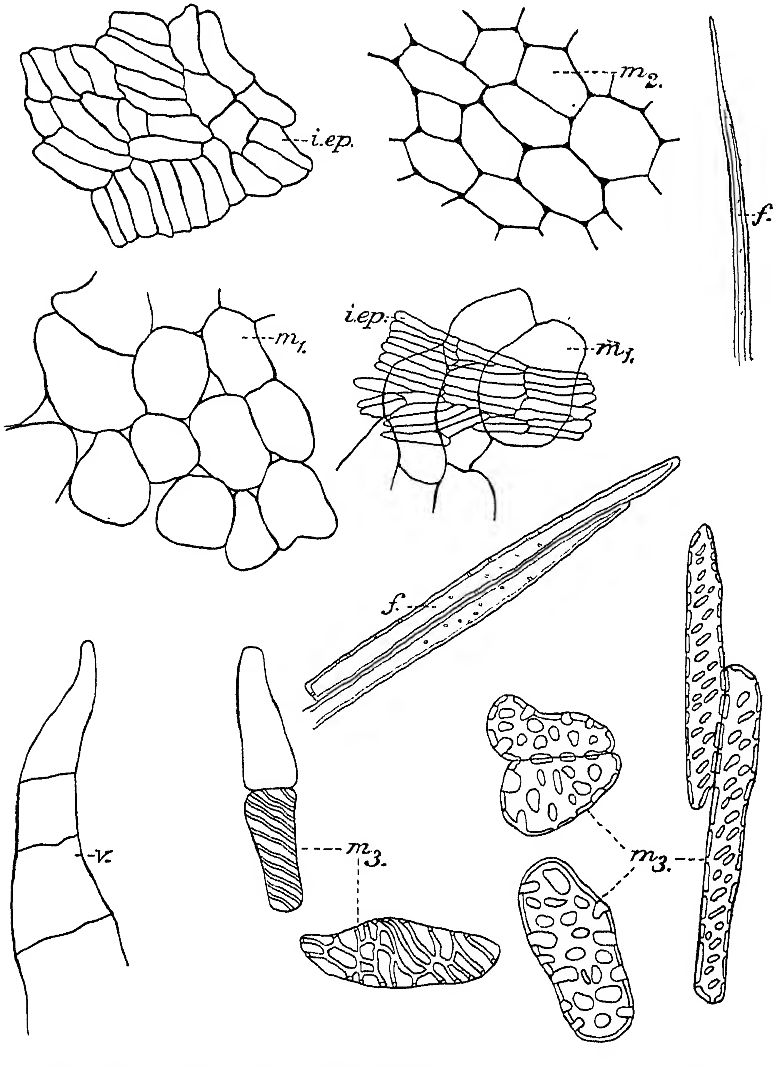

Fig. 159.—Fennel Fruit, tissues separated by digestion with solution of potassium hydroxide. *f*, sclerenchymatous fibres; *i. ep.*, inner epidermis of pericarp; m_1., adjacent parenchyma of mesocarp; m_2., dark-brown parenchyma of mesocarp; m_3., pitted, reticulate, and spiral cells of mesocarp. ×240. *v.*, portion of vitta. ×50.

recognised; they vary in shape from rounded to strongly elongated.

In the preparation from the ridges, abundant reticulated and porous cells of the mesocarp, as well as fibres from the bundles, are to be found, the gradation from a rounded porous cell to a typical sclerenchymatous fibre being quite gradual.

Examination of the Powder.—Dry some of the fruits at about 80° C., powder and sift through a No. 80 sieve, taking care that the whole passes through. Mix a little with dilute glycerin and with solution of chloral hydrate and set aside for twenty-four hours or longer, allowing the water to evaporate slowly from the dilute glycerin preparation.

Moisten a little of the powder with alcohol, add dilute glycerin and examine under the low power. Most of the fragments, especially the smallest, are colourless or greyish, but some are brownish or dark brown in colour.

Stain a little with picric acid and mount in glycerin as directed on p. 192. Examine the smallest particles for aleurone grains, which may be found, although they are minute and not conspicuous; after treatment with dilute solution of potash look for the characteristic rosettes of calcium oxalate. In this preparation radiating tufts of large crystals (of anethol) may occasionally be found.

Examine the glycerin preparation, which is usually clear. Fragments of the endosperm, readily recognised by the greyish colour and thick cell walls, are numerous; the cells contain the characteristic aleurone grains. Small fragments, consisting of portions of adjoining cells and even of portions of the walls only, recognisable by their thickness, are readily found. Portions of the dark-brown vittæ, and of the bundles of fibres from the ribs, are also easily identified, but are better seen in the following preparation.

Examine the chloral hydrate preparation. The walls of the endosperm cells have swollen and the fragments are clearer; the ground substance of the aleurone grains has dissolved and the rosettes of calcium oxalate are distinct; there are also numerous large and small oil globules from the endosperm. Portions of the bundles of fibres are to be detected without difficulty; examine them carefully and note the elongated, pitted or reticulated cells which are often attached to them. Groups of a few fibres and isolated broken fibres are also to be found, but the reticulated cells are not conspicuous. Very conspicuous by reason of their dark reddish-brown colour are fragments of the vittæ with attached, often pigmented, parenchyma, and also the inner epidermis of the pericarp with its characteristic narrow cells. The thin-walled cells of the mesocarp, colourless or brown, are less readily seen, and fragments of the epidermis are rare.

Defat a little of the powder with ether-alcohol, dry, and mount in solution of chloral hydrate. This preparation is free from oil globules and is the clearest of all; it may be utilised for further examination for the inner epidermis of the pericarp and for the reticulated cells. Attention may be directed to the latter by staining a portion of the defatted powder with phloroglucin and hydrochloric acid.

Wheat

Source.—The fruit of *Triticum sativum*, Lam., and other species of *Triticum*.

Preparation.—Examine a grain of wheat with a lens; it is ovoid in shape. One side is rounded, while on the other there is a deep furrow. Near the apex of the fruit there is a tuft of hairs.

Cut a grain longitudinally and examine the section with the lens; near the base the embryo can be seen.

Transverse Sections.—Soak some grains in water until sufficiently soft to cut (twelve hours or more; they may be preserved, after softening, in a mixture of equal parts of 70 per cent. alcohol and glycerin); embed one in pith, and cut a number of transverse sections, taking care that the pericarp of the grain is cut as thinly as possible. Transfer these sections to alcohol. Examine one in dilute glycerin: the abundant starchy endosperm is surrounded by several narrow layers of cells varying from yellow to brown in colour; these layers comprise the tissues of the pericarp and the closely adherent seed coats.

Mount one in chloral hydrate, warming gently to clear it. Examine carefully under the high power, commencing with the outermost layer (epidermis of the pericarp) and passing to a narrow dark-brown line (seed coats), which is usually very distinct, and, finally, to the large thin-walled cells of the endosperm.

The three, or sometimes four, outermost layers of cells are often more or less collapsed to form a pale-yellow tissue, in which indications only of cell cavities can be detected. If this is the case, they must be expanded by warming in chloral hydrate or in potash, but it must be remembered that the use of the latter reagent may cause considerable swelling of the walls.

The epidermal cells are oblong, and possess thickened tangential, but thin radial walls (fig. 160, 1 and 10,$_1$); the next two rows of cells, constituting the hypoderma, are similar in appearance, and both these and the epidermal cells have lignified walls. Pits are difficult to see in the transverse section. Next to this tissue is a row of parenchymatous cells which are larger and have thin walls. These cells are also more or less collapsed, and appear not to be present in every part of the section; they are often difficult to see in transverse sections (fig. 160, 10,$_4$).

Following upon this layer is a single row of (in transverse section) elongated cells that exhibit conspicuous pitting. They average about 110 to 130 μ in length and 12 to 15 μ in width; the walls are yellowish in colour and lignified (fig. 160, 10,$_5$).

Between this and the seed coats are tubular cells which appear as rounded or flattened rings in transverse section. These cells are not regularly arranged, but distributed in an irregular manner, or, at least, are only to be seen at irregular distances, and best in sections that have been expanded by warming in potash. They are the remains of the inner epidermis of the pericarp, the constituent cells of which are tubular in shape and have become separated from one another during the development of the fruit; hence their irregular distribution (fig. 160, 10,$_6$).

The narrow brown line that represents the collapsed seed coats is often apparently homogeneous, but under the influence of caustic potash indications of radial walls can often be detected (fig. 160, 10,$_7$).

Abutting upon the seed coats are the remains of the nucellus, which appear as a narrow hyaline layer. After warming in chloral hydrate, the tangential walls of the constituent cells swell and exhibit distinct striations; the radial walls also become visible. Two rows of such cells can be seen (fig. 160, 10,$_8$).

There follows next the aleurone layer, a single row of cells which constitutes the outermost layer of the endosperm (fig. 160, 10,$_9$). The cells of which it consists are large (45 to

60 μ by 30 to 50 μ) and radially elongated. The walls are rather thick. The cells are packed with small rounded aleurone grains (picric acid reaction on a fresh section), associated with oil-containing plasma (sulphuric acid reaction). The cells of the endosperm are large and have very thin walls; they are filled with starch grains (see p. 14) associated with minute aleurone grains (fig. 160, $10, _{10}$).

Examine the furrow that runs down one side of the grain; in the sections this will of course be cut transversely. The seed coats can easily be traced by reason of their brown colour; they follow the course of a W. The space of the central triangle is occupied by thin-walled parenchymatous tissue, apparently an expansion of the tissue of the nucellus. Here the aleurone layer has often broken away from the nucellus. At the apex of the triangle there is a group of thin-walled cells filled with a brown amorphous substance; these cells are part of the seed coats.

The two lateral triangles of the W are occupied by collapsed cells derived from the outer layers of the pericarp; the sclerenchymatous cells can be traced nearly to the apices of these triangles; they resemble the corresponding cells from other parts of the grain.

Radial Sections.—Cut next longitudinal sections. The pericarp exhibits a structure similar to that observed on the transverse section, but the epidermal and hypodermal cells are small and exhibit an almost isodiametric section; the cells of the seed coats are often a little more distinct.

At the base, on the curved side of the fruit, is the embryo. This is built up of small thin-walled cells filled with plasma and oil.

Near the apex of the grain there are numerous long, one-celled, thick-walled, tapering hairs which must be closely examined; they can be better seen in a surface preparation.

Surface Sections.—Next cut surface sections from several fruits; immerse them in alcohol and then examine in dilute glycerin or chloral hydrate, taking care that the epidermis is uppermost. Focus down through one layer to the next, as may be necessary.

The first layer is the *epidermis*; it consists of elongated cells (often 100 to 200 μ long and 25 to 50 μ wide), with rather thickened pitted longitudinal walls, but thin transverse walls, the latter being often more or less oblique, so that the cells are somewhat pointed (fig. 160, 1).

The second layer consists of similar cells, but the transverse walls are often more distinctly pitted than they are in the epidermal cells (fig. 160, 2).

The third layer is composed of parenchymatous cells with thin walls, which are not pitted ; this layer is very difficult to see.

The fourth layer consists of irregularly shaped parenchymatous cells with large intercellular spaces and coarsely pitted walls ; it is best seen in a subsequent preparation, in which it is generally found adhering to the upper surface of the fifth layer ; in places this layer is interrupted (fig. 160, 3 and 4).

The fifth or *sclerenchymatous layer.*—This layer is usually very conspicuous in chloral hydrate ; the cells are elongated transversely to the epidermal cells ; their walls are thickened and conspicuously pitted ; the layer is continuous, and exhibits but few and small intercellular spaces (fig. 160, 5).

The sixth layer (or *tubular cells*) is often difficult to find, and is better seen in maceration preparations. The cells are narrow tubes, between which are often large spaces ; the walls are pitted where they adjoin other similar cells. They are arranged transversely to the sclerenchymatous layer (fig. 160, 6)

The seventh layer comprises the seed coats ; it is pale yellow in colour, and is composed of two layers of elongated thin-walled parenchymatous cells crossing one another at right angles (fig. 160, 7).

The eighth layer, or nucellus, is difficult to find ; it is best

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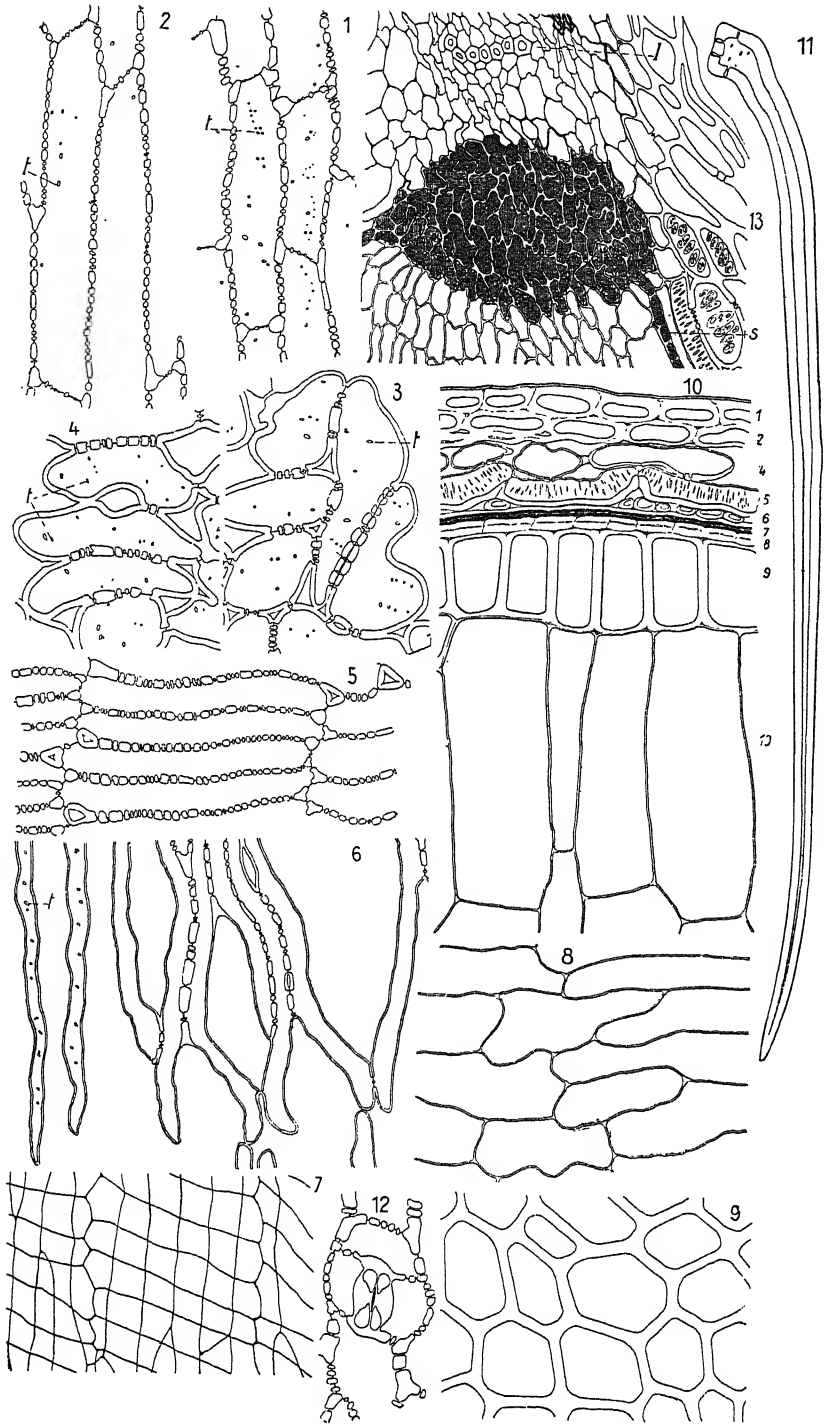
Fig. 160.

1. Epidermis of pericarp, surface view; *t*, pits on inner wall.
2. Hypoderma of pericarp.
3. Middle layer of pericarp (4 in fig. 10), usual form of cell ; 4, unusual form of the same cells.
5. Transverse cells of pericarp, surface view.
6. Cells of the inner epidermis of pericarp, surface view.
7. Cells of seed coats, surface view.
8. Cells of nucellus (after treatment with potash).
9. Cells of epidermis of endosperm (aleurone layer).
10. Transverse section of the pericarp, seed coats, and part of the endosperm of the grain ; 1, epidermis ; 2, hypoderma ; 4, middle layer ; 5, transverse cells ; 6, inner epidermis of pericarp ; 7, seed coat ; 8, nucellus ; 9, aleurone layer ; 10, endosperm.
11. Hair from apex of fruit.
12. Stoma from apex of fruit.
13. Section through the furrow ; *b*, vascular bundle ; *s*, seed coat.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examined in sections that have been warmed in chloral hydrate (fig. 160, 8).

The ninth layer is the aleurone layer; the cells are thick-walled, nearly isodiametric, and they are filled with densely packed aleurone grains (fig. 160, 9).

The *endosperm* consists of large thin-walled cells filled with starch grains (fig. 160, 10, $_{10}$).

Maceration Preparations. In addition to the sections and surface preparations it is very desirable to separate the layers of cells as much as possible from one another, and to scrutinise and identify them. For this purpose the following method is an excellent one, although it requires considerable time; it has the advantage of allowing the tissues to be separated without causing any swelling or other appreciable alteration in the walls. It consists in simply crushing the grains and allowing them to stand, covered with water, in a warm place (20° to 40° C.); putrefactive decomposition sets in, and, after a few days, the layers can be separated by teasing; they may be examined in dilute glycerin. In this preparation carefully examine the hairs. They vary considerably in length; many are between 200 and 500 μ long, but occasionally they are still longer. The walls are thick and the lumen narrow; towards the apex the latter becomes gradually narrower, towards the base larger, but at the base itself it enlarges rather abruptly, and here the wall is distinctly pitted. The diameter at the base is about 18 to 24 μ. In most of the hairs the wall is as thick as, or thicker than, the cavity of the cell.

Having thus thoroughly studied the anatomy of the grain, the student may proceed to study the powder, which should be prepared from dried grains and passed through a No. 60 or No. 80 sieve, taking care that the pericarp of the fruit, or part of it at least, passes through the sieve.

The starch may be examined in water as usual, and the minute aleurone grains detected with picric acid. The epidermis and aleurone layer can be well seen after soaking in dilute glycerin, but for the examination of the other fragments of pericarp chloral hydrate is better; allow the preparation to stand until the starch is gelatinised, or effect this by gently warming. The sclerenchymatous cells are generally particularly conspicuous, and to this layer the seed coats and nucellus adhere on the under side, while the upper often bears the interrupted layer of parenchyma (layer 4). The hairs are fairly numerous and readily identified. Most of the tissue found in the examination of the grain may be recognised with facility.

Finely sifted flour consists to a large extent of starch, but it always contains aleurone grains, and also fragments of the pericarp (bran). The more efficient the sifting of the flour, the fewer and smaller will these fragments be. They are, however, important adjuncts in identifying the flour, and must be collected and examined.

They are best collected as follows:

Mix 5 grammes of flour with 50 c.c. of water, add about 2 c.c. of hydrochloric acid (sp. gr. 1·16), and bring the mixture to the boiling-point; boil gently for five to ten minutes. Cool, and add sufficient solution of potash to make the liquid slightly alkaline; it will then become nearly clear. Divide this liquid among the tubes of a centrifuge,[1] and separate the deposit. Collect it in one tube, wash once with water; mount slides of this deposit in water, dilute glycerin, and chloral hydrate respectively. Numerous hairs will be found, as well as small fragments of the pericarp, seed coats, &c., together with various impurities that may be present. The tissues derived from wheat can easily be examined and identified; those foreign to wheat can readily be detected.

Diagnostic Characters of other Flours

Vogl[2] gives a useful key for the identification of the various flours, from which the following details are taken.[3]

Starch Grains

A. Large grains and very small grains, with but few of intermediate size. Large grains simple, flattened; in side view elliptical or lens-shaped; in surface view circular.

Wheat. Large grains 36 to 39 μ in diameter, isolated grains attaining 45 μ; in surface view circular or slightly reniform; at most a grain here and there with distinct striations and a cleft or stellate hilum.

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[1] If a centrifuge is not available, subsidence in a urine glass may be resorted to.

[2] *Nahrungs- und Genussmittel*, p. 153.

[3] For illustrations of the powders of various flours see Greenish and Collin, *Anatomical Atlas of Vegetable Powders*.

Rye. Large grains 36 to 47 μ in diameter, isolated grains attaining 52 μ; often with very distinct striations and cleft or stellate hilum.

Barley. Large grains 18 to 30 μ, mostly 21 to 28 μ, in diameter. In surface view less regularly circular than wheat; often a little irregular in outline, exhibiting depressions or protuberances, broadly reniform or bean-shaped, or with three or four rounded angles.

B. Large and small spheroidal compound grains, isolated components of the same, and also simple grains.

Oat. Component grains 3 to 7 μ in diameter, angular or with rounded angles; simple grains (3 to 7 μ), associated with very small compound grains with two or three components. Shape variable; particularly noticeable are crescent-shaped, pointed-elliptical and lemon-shaped grains.

Rice. Component grains angular, or with slightly rounded angles; simple grains polyhedral, mostly exhibiting in surface view five to six sides, almost regular and tolerably uniform, 3 to 9 μ in diameter (mostly 6 μ); most grains exhibiting a conspicuous cavity at the hilum.

C. Simple polygonal grains with sharp or rounded angles, together with some rounded grains.

Maize. Grains 6 to 21 μ, mostly 12 to 18 μ, in diameter; sharply polygonal, some with rounded angles; other grains rounded; often with large hilum or stellate cavity. The grains are sometimes cemented into groups by means of a thin layer of protoplasm, which gives place to a number of minute granules when treated with caustic potash.

Millet. Grains 4 to 12 μ, sometimes 15 μ, seldom showing an angular or stellate cavity; a few smaller, rounded, ovoid, or spindle-shaped. Mounted in water, there is no distinct layer of protoplasm visible.

Buckwheat. Grains 6 to 12 μ, at most 15 to 18 μ, the majority 9 to 12 μ; sides often concave, less often sharply and uniformly polygonal; some of the groups of cells have remarkable shapes, elongated, club-shaped, curved, &c. Mounted in water, there is no distinct layer of protoplasm visible.

Fragments of Tissue

A. Remains of the paleæ present, with characteristic epidermis.

Barley or Oat.

B. Fragments and tissues of the pericarp exhibit the following characters:

(*a*) **Epidermis.**

Wheat. Cells with four to six straight walls, the lateral walls exhibit fairly regular thickening.

Rye. Cells similar, but the lateral walls exhibit irregular thickening.

Barley. Cells thin-walled, exhibiting not more than indications of thickening: stomata and hairs present (see under *b*).

Oat. Cells thin-walled, with hairs (see under *b*).

Maize. Cells with sinuous lateral walls; length up to 180 μ; width, 15 to 30 μ; coarsely pitted, thick or thin walled.

Millet. Cells with sinuous lateral walls, 30 to 120 μ long, 14 to 30 μ wide.

Rice. Cells with sinuous transverse walls, 60 to 75 μ long, 7·4 to 24 μ wide; the sinuosities mostly on the short walls.

(*b*) **Hairs.**

Wheat. Up to 1 mm. long; the wall as wide as or wider than the lumen; often bent above the basal part.

Rye. Similar to wheat, but the walls narrower than the lumen and the hairs not bent.

Barley. Thin-walled, conical, or trumpet-shaped, 30 to 180 μ long, 9 to 21 μ wide.

Oat. Usually two or three together, straight, very long, and gradually tapering.

(*c*) **Transverse Cells.**

Wheat. Single layer of thick-walled cells with almost straight longitudinal walls, which are rather regularly pitted and exhibit no intercellular spaces.

Rye. Similar, but the ends are often rounded and strongly thickened; with intercellular spaces.

Barley. Two rows of thin-walled cells with abu
intercellular spaces.

Oat. Single layer of cells, their long axes often oblic
the epidermal cells.

Rice. Cells narrow; strongly tangentially elongate
always closely abutting on one another.

(*d*) **Tubular Cells.**

Wheat, rye, barley. Cells often thick-walled, 15 to
wide, scattered or irregularly distributed.

Rice, millet, maize. Cells very numerous, close tog
mostly 3 to 5 μ wide (in maize two rows crossi
another).

C. Cells of aleurone layer exhibit the following charac

Wheat. Cells in a single row, very thick-walled; in s
almost quadratic.

Rye, oat, maize. Cells in a single row, thick-walle
section radially elongated.

Rice, millet. Cells tend towards tangential elong
relatively thin-walled, not collenchymatous.

Buckwheat. Cells similar, but rather thick-walle
collenchymatous.

Barley. Two or three rows of cells.

Examination and Identification of Powdered Fruits
methods adopted for the examination and identifica
powdered fruits are the same as those for powdered see
is frequently very difficult to distinguish seeds from
but the presence of empty parenchymatous cells, of
in any abundance, or of fragments of epidermis wi
obliterated stomata, usually indicates a powdered fruit.

SECTION XII

RHIZOMES

INTRODUCTION

In its widest signification the term ‘ rhizome ’ includes all hypogæic or epigæic stem formations which differ from aerial stems in their shape, appearance, size, duration and structure. They are usually thickened and horizontal or nearly so, but they may be slender and may assume temporarily or permanently an oblique or erect position. Sometimes the branches only of the rhizome ascend and produce leafy and flowering stems, while the rhizome itself continues its growth. But more frequently the axis itself curves upwards and elongates into an aerial stem. This stem is annual, and, when its period of growth is completed, it perishes. This would cut short the life of the rhizome were it not for the fact that a bud in the axil of one of the cataphyllary leaves borne by the rhizome and its branches develops and continues the life of the rhizome, forming simultaneously a sympodial branch system.

The structure of the typical horizontal rhizome, such as that of podophyllum or arnica, is analogous to that of aerial stems.

The stele is separated from the cortex by an endodermis, which is often easily discernible under the microscope. The tegumentary tissue is usually cork, the epidermis having in most cases been thrown off. The tissue which has been designated ‘ outer bark ’ (see p. 150) is comparatively seldom produced. The cortex is often largely developed, and is obliquely traversed by leaf traces passing into the cataphyllary leaves. In the stele of dicotyledonous rhizomes the wood bundles may be arranged in a close or diffuse ring ; here also the parenchyma is often largely developed and filled with reserve material.

Most of the officinal rhizomes are obtained from monocotyledonous plants. In these the bundles are closed and no cambium is formed. Very frequently each bundle is supported by a crescent-shaped mass of sclerenchymatous fibres. In those rhizomes in which the internodes are very closely approximated, these bundles often assume an oblique or even nearly transverse direction.

As there is by no means so great a differentiation of the tissue in subterraneous organs as in aerial, the tissues themselves of rhizomes offer fewer diagnostic characters than those of aerial stems, and it becomes necessary to pay very careful attention to the minuter details.

Most rhizomes serve as organs for the storage of reserve materials, and the parenchymatous tissue destined to receive these is therefore largely developed. During the process of pulverisation these cells, being usually thin-walled, are more or less completely broken up, and hence the powder consists largely of the reserve material which they contained and of the *débris* of the cell walls. Concerning the reserve materials, sufficient has already been said in the preceding chapters, and the student will be acquainted with the diagnostic importance of starch, calcium oxalate, &c.

Particular attention must be paid to the nature of the walls of the parenchymatous cells, their thickness, the size and distribution of the pores, &c., as by these means the parenchyma of one drug may often be distinguished from that of another. The vessels and fibres must be isolated, and their shape, colour, thickness of wall, and pores carefully studied.

The examination of the powdered drug is best conducted first in water or dilute glycerin, in which the colour of the tissues present, the details of the cell walls, of starch, &c., may be well observed. Boiling with dilute solution of potash, which is subsequently washed out with water, often clears the powder from cell contents, and leaves the cell walls practically unaltered. Digestion with chloral hydrate often answers the same purpose, the powder being separated from the liquid by a centrifuge. Dissociation of the elements, which is often necessary, may be effected by maceration with potassium chlorate and nitric acid, while, for very starchy rhizomes, boiling with dilute hydrochloric acid followed by dilute potash, as detailed under wheat flour, yields excellent results, the details of the vessels and fibres being usually very clear.

Arnica Rhizome

Source.—The rhizome of *Arnica montana*, Linn.

Examination of Sections.—Select two or three pieces of arnica rhizome, breaking them to see that they are sound. Soak them twelve hours in water, and then expose them to the air until they assume a tough condition in which they are suitable for cutting. Cut transverse sections, and transfer them to alcohol.

Mount and examine one in chloral hydrate. Observe the circle of irregular wood bundles; within the circle is the pith; beyond it are the bast ring, cortex, and tegumentary tissue (fig. 161). In order that the last-named three tissues may be correctly described, it is necessary that the position of the endodermis—which is the innermost layer of cortical cells and consequently separates the cortex from the bast ring—should be determined. This is possible with arnica, and also with many other, but not all, rhizomes.

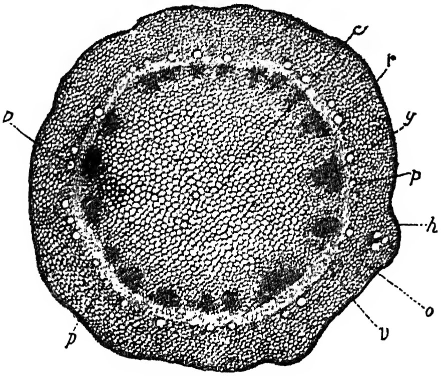

FIG. 161.—Arnica Rhizome, transverse section. *c*, pith; *o*, cortex; *p*, oleoresin ducts; *r*, medullary rays; *v*, bast; *y*, wood bundles. ×10. (Berg.)

In arnica rhizome the endodermis can usually be found without special treatment. Observe, between the tegumentary tissue and the wood bundles, a number of large schizogenous oleoresin ducts arranged in a circle. The endodermis is just within this circle; indeed, it often curves round the inner margins of the ducts, thereby assuming a sinuous course. The cells of which it is composed are narrow, tangentially elongated, and characterised by distinct suberisation and lignification in the centre of the radial walls (compare Lobelia Stem, p. 81). This lignified and suberised portion is strongly refractive, and hence easily visible. Should it not be apparent, it can be made more conspicuous by staining it with phloroglucin and hydrochloric acid.

Having determined the position of the endodermis, make a diagrammatic sketch of the section.

Examine now in succession the tissues of which the rhizome consists, commencing with the outside.

(1) **Tegumentary Tissue.**—If the cells of which this is composed are not easily seen in chloral hydrate, and this is often the case, mount a fresh section in solution of potash, warm it gently, and cool; they will then be distinctly visible; the tissue consists of several rows of cork cells, with thin brown walls.

Examine a very thin section in water; these cells (or at least some of them) have brown amorphous contents. Irrigate the section with dilute solution of ferric chloride; the brown changes to greenish black (tannin reaction).

(2) **Cortex.**—The tissue from the phellogen up to and including the endodermis is cortex. Careful examination will show that no secondary cortex (phelloderm) has been formed. The cortical parenchyma consists of cells with slightly thickened pitted walls; most of the cells are somewhat tangentially elongated, and near the phellogen are often of collenchymatous nature. Near the endodermis there is a diffused circle of large

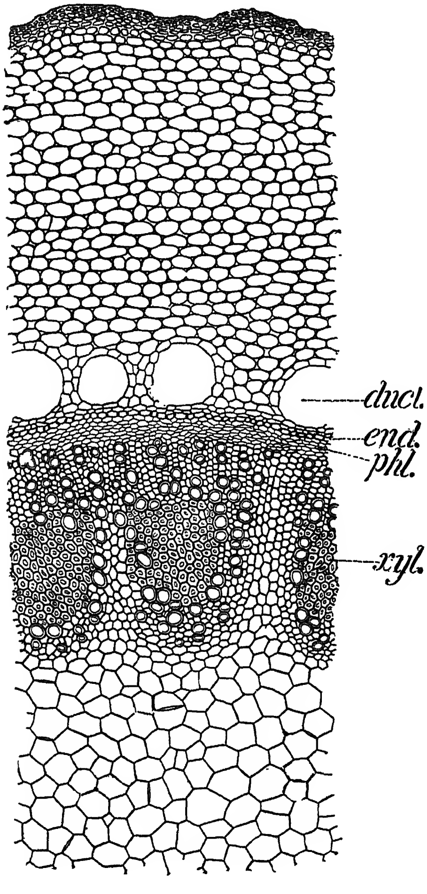

FIG. 162.—Arnica Rhizome, transverse section. *end.*, endodermis; *phl.*, phloem; *xyl.*, xylem. ×80. (Partly after Berg.)

oleoresin ducts of obviously schizogenous origin. These ducts generally exhibit a well-defined tapetal layer, from the inner cell wall of which little gelatinous papillæ project into the cavity of the duct. These papillæ are the remains of the resinogenous layer of the cell wall; in this layer the oleoresin is formed, and from it the secretion is discharged into the cavity of the duct (Tschirch). Similar but smaller ducts may also be observed in the cortex, nearer the cork, accompanying in pairs small fibro-vascular bundles (leaf traces).

The cells of the cortex contain small granules. Some of these stain yellow with iodine, and are doubtless granular remains of the protoplasm. Here and there large amorphous or indistinctly crystalline lumps of inulin are to be seen (compare the characters of this substance detailed under Taraxacum Root). Tincture of alkanna shows the presence of but little oil. With concentrated sulphuric acid fairly abundant acicular crystals of calcium sulphate are obtained, indicating the presence of notable quantities of this metal.

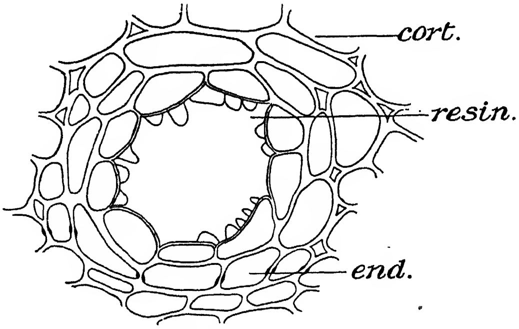

FIG. 163.—Arnica Rhizome, transverse section of oleoresin duct, showing the resinogenous layer. ×250.

The bast ring is narrow, and consists of small elements that do not call for special note. In the wood bundles the centre is generally occupied by a mass of wood fibres, often of considerable extent. The pith is composed of rather large parenchymatous cells, with intercellular spaces; in these cells lumps of inulin may often be found.

Ginger

Source.—The rhizome of *Zingiber officinale,* Roscoe.

Examination of the Rhizome.—For examination select a piece of unbleached Jamaica ginger from which the cork has been removed by scraping. Break the rhizome transversely between two of the branches, smooth the broken, transverse surface with a knife, and examine.

Observe the large stele, which is surrounded by a distinct line, usually designated the endodermis; the cortex is comparatively narrow. Throughout both stele and cortex there are scattered dark bundles and pale yellowish-brown secretion cells.

Transverse Sections.—From the dry transverse surface cut several sections, taking care to cut from the stele as well as from the cortex, and to include, therefore, all tissues from the margin to the centre. Transfer them to alcohol, bearing in mind, of course, that the oleoresin will be dissolved; direct attention at first to the structure, and leave the cell contents to be studied later with due precautions.

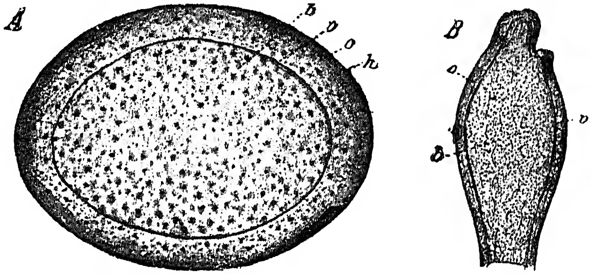

FIG. 164.—Ginger Rhizome. *A*, transverse section × 3; *B*, radial section, natural size. *o*, cortex; *v*, endodermis; *b*, stele; *h*, cork (or outer portion of cortex). (Berg.)

Mount a section in water and examine under the low power; there is so much starch present as to obscure the structure.

Mount another in chloral hydrate and gelatinise the starch by warming. The structure becomes more distinct. The cortex is free from tegumentary tissue of any kind (this has been removed by peeling). It consists of thin-walled parenchymatous cells, with intercellular spaces. This tissue contains scattered oil cells, and is traversed by fibrovascular bundles (leaf traces), which are cut transversely, or slightly obliquely.

The inner limit of the cortex (endodermis) cannot be easily identified. Observe towards the interior of the rhizome a pale brownish circle (visible under a lens). On the inner (concave) side of this line a number of fibrovascular bundles can be seen;

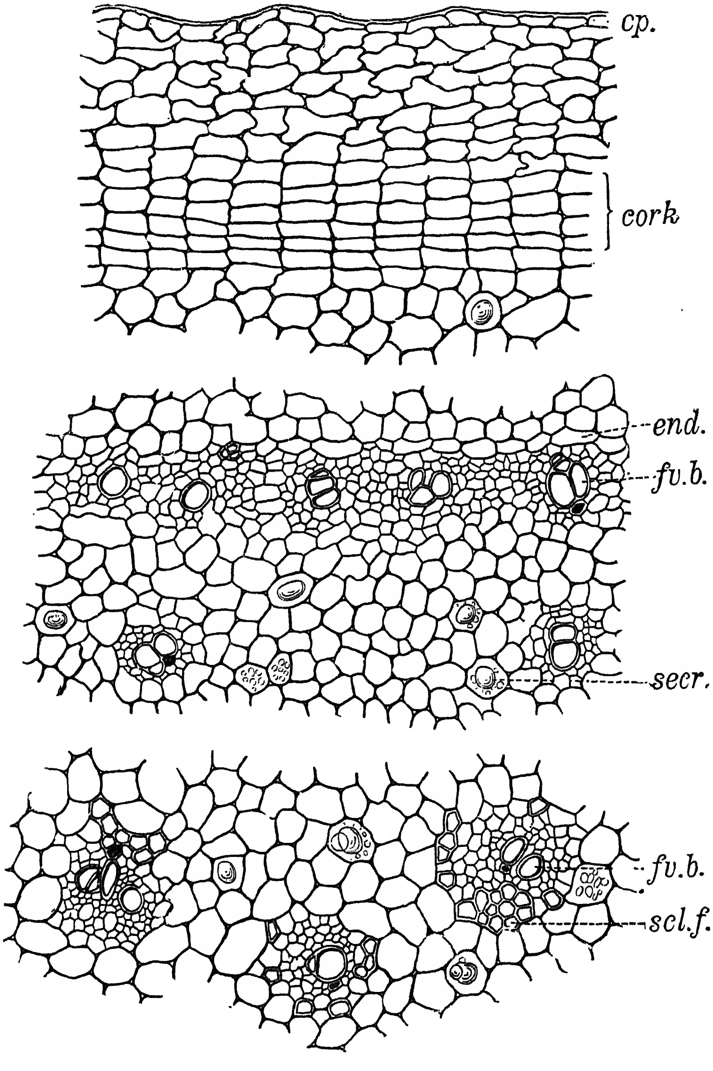

Fig. 165.—Ginger Rhizome, transverse section. *end.*, endodermis; *ep.*, epidermis, which together with the subjacent cork is often removed by scraping; *fv. b.*, fibrovascular bundle; *scl. f.*, sclerenchymatous fibres; *secr.*, secretion (oleoresin) cells. (Tschirch and Oesterle.)

the structure of the line itself is not very distinct, the cells being more or less collapsed. Mount another section in concentrated

(25 to 50 per cent.) solution of potash; warm gently, not quite to boiling. The walls of the collapsed cells expand, and the endodermis can be recognised by its strongly refractive radial walls. Within the endodermis, between it and the circle of bundles, are two (or three) rows of tangentially elongated parenchymatous cells, but the radial walls of these cells are not strongly refractive. Wash out the solution of potash with water; irrigate with concentrated sulphuric acid. The radial walls of the endodermis do not dissolve; they are suberised.

The bundles immediately within the endodermis are tangentially elongated, and abut so closely upon one another as to form an almost continuous circle. The remainder of the stele consists of thin-walled parenchymatous cells, with scattered oil cells, and more or less rounded fibrovascular bundles.

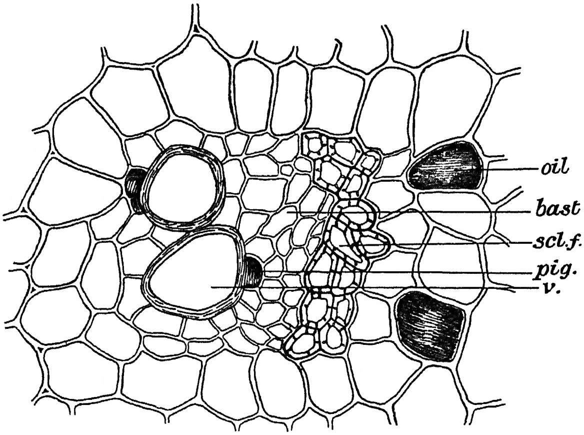

FIG. 166.—Ginger Rhizome, transverse section, through a bundle, with neighbouring parenchyma. *oil*, oleoresin cells; *pig.*, pigment cells; *scl. f.*, sclerenchymatous fibres; *v.*, vessel. (Vogl.)

Examine these bundles more carefully. They are collateral, and consist of a few vessels, mostly of considerable size, abutting upon bast tissue, the whole supported or surrounded by fibres with thickened walls. In some of the bundles one or sometimes two small cells filled with a brown homogeneous amorphous substance can be seen adjoining one of the vessels.

Cut and examine longitudinal sections of the rhizome, selecting those that pass through the bundles. The parenchymatous cells are axially elongated. The vessels are spiral, reticulated or scalariform (or intermediate between these).

The fibres have rather numerous elongated or cleft pits arranged in a left ascending spiral.

The brown substance in the small cells above referred to is soluble in solution of potash and in chloral hydrate, and may therefore escape observation. Mount a fresh section in water, and remove this by filter paper; drop on concentrated hydrochloric acid, cover, and gently warm to expel air bubbles. The secretion cells are now very conspicuous; they are narrow elongated tubular cells, which, however, are not continuous, and therefore are not seen in the transverse section of every bundle. These secretion cells are characteristic of Zingibereæ. In this preparation numerous oil cells containing the oleoresin may be found.

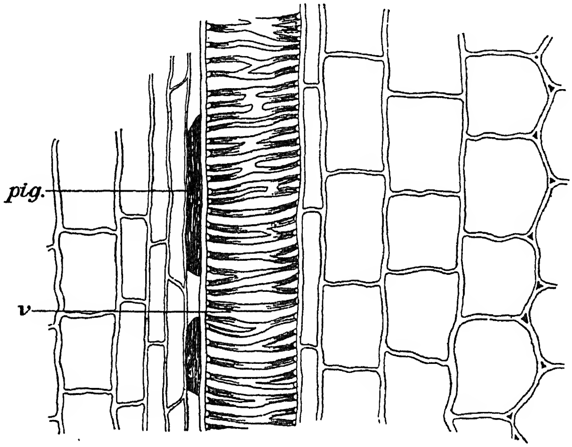

FIG. 167.—Ginger Rhizome, radial section through a portion of bundle. *pig.*, pigment cells; *v*, vessel. (Vogl.)

Isolate some of the fibres and vessels by maceration with potassium chlorate and nitric acid, and examine. The fibres vary both in length and shape. Some taper at the ends, others are rounded, others again are square. Sometimes they exhibit thin transverse divisions. The pits are not easily seen after the treatment with nitric acid; they are better studied in longitudinal section (see above).

Examine next the starch grains. Many of them are sack-shaped, ovoid or elongated-ovoid, flattened and more or less pointed at one end, but rounded at the other, the hilum being close to the apex of the pointed extremity and so eccentric as to be almost invisible ; the smaller grains are often nearly circular. The striations are distinctly visible, especially in the larger grains, when carefully illuminated.

The grains vary mostly from 20 to 30 μ in length, but some measure as much as 40 μ, while occasionally 50 μ is reached ; the thickness is generally from 5 to 10 μ.

Proceed now to determine the distribution of the oleoresin cells. Cut a moderately thick transverse section, mount in glycerin, or dilute glycerin, and gently boil to expel air and to gelatinise the starch. Examine under a low power; the oleoresin cells are scattered throughout the cortex and stele ; they contain a pale-yellow oleoresin.

A section similarly treated with solution of caustic potash also shows the oleoresin cells, but they will now contain a deep reddish-brown liquid in which globules of oil are visible, the resin having combined with the alkali to form a dark-coloured compound in which the globules of oil remain suspended.

Powdered Ginger

Examination of the Powder.—The student should now examine powdered ginger, with the object of identifying in it all those structural elements and cell contents that have been observed in the rhizome.

Moisten a little powdered ginger with water ; allow it to stand a few hours, and then examine. The preparation contains abundance of starch grains ; examine these carefully, and compare with the starch grains of the rhizome ; they often appear granular from adhering particles of proteid matter, &c., from which they can be partially freed by mounting in very dilute (0·1 per cent.) solution of potash. Most of the cells with yellow or brownish oleoresin are destroyed by the grinding, but a few remain intact. The sclerenchymatous fibres are pale yellowish in colour.

To examine the sclerenchymatous elements present, it is desirable to get rid of the starch, and thus concentrate the vessels, fibres, &c., into a smaller compass. This may be done as follows :

Mix 5 grammes of the powdered ginger with 50 c.c. of water and add 2 c.c. of the official hydrochloric acid (sp. gr. 1·16). Raise to the boiling-point, and boil gently for ten minutes. Then centrifuge for two minutes ; this suffices to separate all the sclerenchymatous tissue and most of the parenchyma. Wash once with water, separating again by the centrifuge. Stir the residue in a few cubic centimetres of solution of chloral hydrate and again separate by the centrifuge, pour off the chloral hydrate, and examine the deposit either in that medium or in glycerin. If a centrifuge is not at hand, the deposit obtained from the acid liquid may be washed with very dilute (about ·5 to 1 per cent.) solution of potash, then once with water, and finally mounted in solution of chloral hydrate.

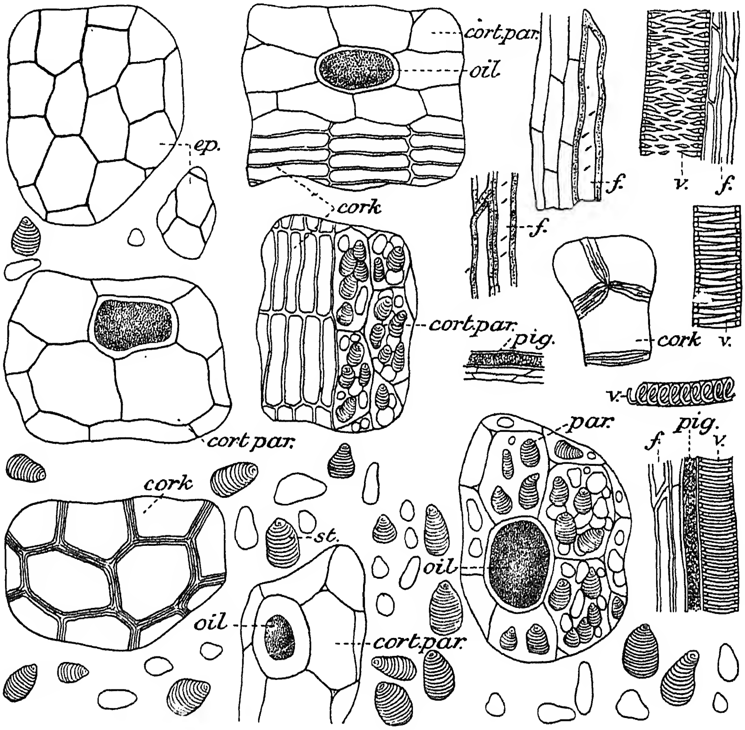

FIG. 168.—Powdered Ginger. *cork*, cork, in transverse section and surface view; *cort. par.*, cortical parenchyma; *ep.*, epidermis; *f.*, sclerenchymatous fibres; *oil*, oil or oleoresin; *par.*, parenchyma of stele; *pig.*, pigment cells; *st.*, starch; *v.*, vessel. (After Greenish and Collin.) ×150.

The deposit will be free from starch, oleoresin, and proteid matter; it will consist of the sclerenchymatous fibres, the vessels, and the *débris* of the parenchymatous tissue, all of which may be examined in this preparation.

The fibres exhibit their shape and their left-spiral pits; the vessels are mostly reticulate, and vary from 30 to 70 μ in diameter; here and there the brown secretion cells that abut on them may be detected; the parenchymatous cells have very thin walls, those of the oleoresin cells being suberised; the latter may be detected by staining with Soudan red.

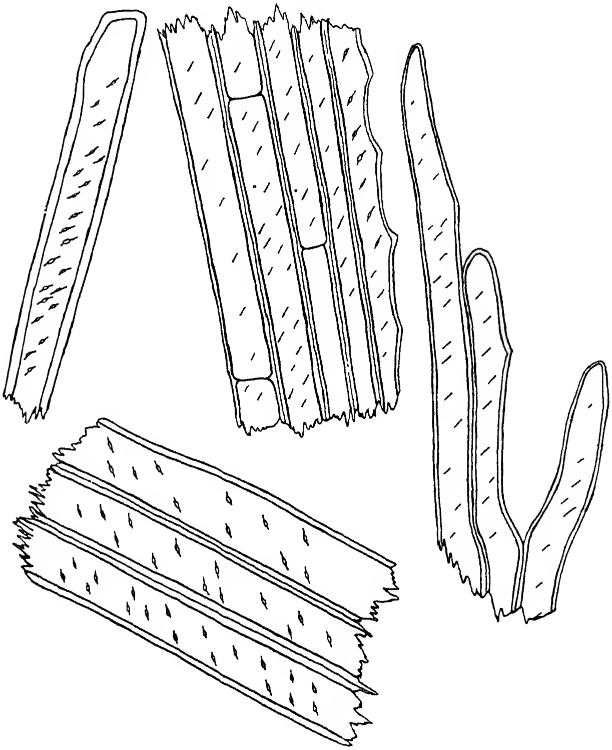

FIG. 169.—Ginger Rhizome, fragments of sclerenchymatous fibres in the powder. ×200.

Galangal Rhizome

Source.—The rhizome of *Alpinia officinarum*, Hance.

Preparation and Examination.—Galangal rhizome may be prepared and examined by the methods recommended for ginger, which drug it resembles in structure. Differences are to be found in the following particulars:

(*a*) The starch grains are rather larger than those of ginger;

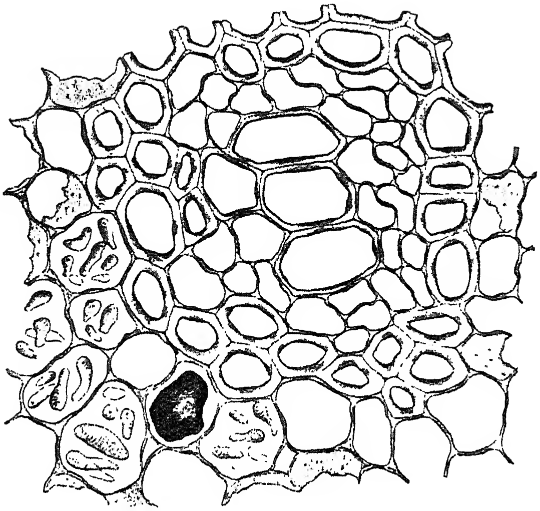

FIG. 170.—Galangal Rhizome, transverse section of a fibrovascular bundle, with adjacent parenchyma; some of the cells contain starch, in one a dark mass of oleoresin. (Moeller.)

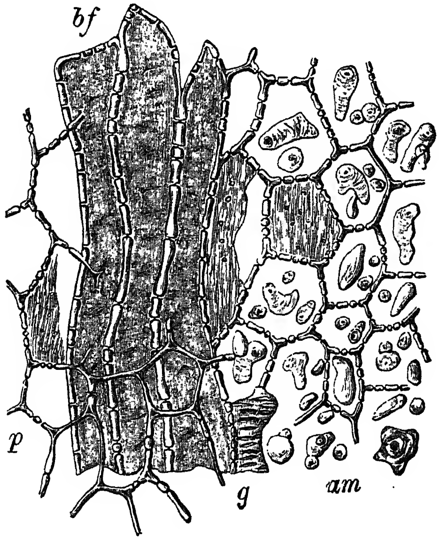

FIG. 171.—Galangal Rhizome, fragment from the powder. *am*, starch; *bf*, bast fibres; *g*, vessel; *p*, thick-walled parenchymatous cells. ×160. (Moeller.)

many are club-shaped, others are reniform, some exhibit irregular protuberances. They are not flattened, as scitaminaceous starches usually are, and are distinctly striated.

(*b*) The parenchymatous cells have thicker walls and are brownish in colour ; the secretion cells are numerous, and contain a dark reddish-brown oleoresin.

(*c*) The bundles are more numerous than those of ginger, and therefore the powder contains more bast fibres and vessels.

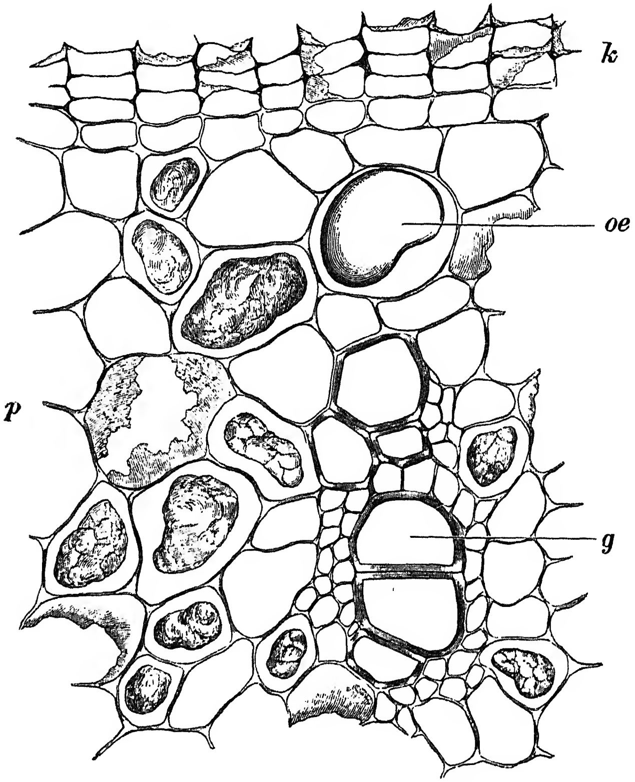

FIG. 172.—Turmeric Rhizome, transverse section. *g*, vessel ; *k*, cork ; *oe*, oleoresin ; *p*, parenchyma of wood, containing masses of gelatinised starch. (Moeller.)

Turmeric

Source.—The rhizome of *Curcuma longa*, Linn.

Turmeric resembles ginger and galangal in structure, but differs from both in the absence of bast fibres. It is further characterised by the yellow colouring matter, which, however, is no longer restricted to particular cells, as it is in the fresh rhizome, but is distributed throughout the drug. This change is due to the scalding to which the rhizome is subjected, and this also causes the gelatinisation of most of the starch grains. Many of the parenchymatous cells, therefore, contain irregular yellow masses of gelatinised starch. Although most of the starch is swollen, here and there grains may be found that still exhibit the characteristic scitaminaceous shape.

The yellow colour is changed to deep red by sulphuric acid, either concentrated or diluted with an equal volume of water or alcohol; in the latter case the red substance produced dissolves. This reaction, which is due to the action of the sulphuric acid upon the curcumin of the rhizome, forms a useful means of detecting powdered turmeric.

SECTION XIII

ROOTS

INTRODUCTION

Nearly all the roots used in medicine are derived from dicotyledonous plants, and hence bear, as might be anticipated, so close a resemblance in structure to dicotyledonous rhizomes that the methods of treatment that have been described for rhizomes are equally applicable to roots. It must be borne in mind that many drugs commonly called roots consist either entirely (rhubarb) or partially (liquorice) of rhizome, and that some roots pass gradually towards the upper part into rhizome (taraxacum), so that a mechanical separation of rhizome from root becomes impossible. While very young roots can without difficulty be distinguished from very young rhizomes by the relative position of the bast and wood bundles, this is by no means the case with older ones, in which this difference has become so obscured as, with few exceptions, to elude detection. Rhizomes frequently bear cataphyllary leaves, the scars of which are often visible in the dry drug ; these leaves are connected with the stele of the rhizome by meristeles that pursue a more or less oblique course through the cortex, and hence are often visible in a transverse section. Roots, on the other hand, are free from such leaf traces. Rhizomes, further, often contain a distinct pith, from which roots are free, but it must be remembered that many roots contain a central parenchymatous tissue practically indistinguishable from the pith of stems.

Monocotyledonous roots differ essentially from dicotyledonous in containing closed instead of open bundles ; they therefore usually exhibit clearly their primary structure.

Belladonna Root

Source.—The root of *Atropa Belladonna,* Linn.

Examination.—Select a small piece of starchy belladonna root about 5 to 7 mm. in diameter; expose it to a moist atmosphere for a few hours. Cut transverse sections, and immerse them in alcohol. Examine one in dilute glycerin. There is an abundance of starch in the cells; examine this later.

Clear a section by solution of potash (or by chloral hydrate), warming, if necessary, to exhibit the cork cells. The cork consists of three or four rows of tangentially elongated tubular cells, on the outside of which there is often a little granular matter (particles of earth) to be seen.

Determine the position of the cambium, which is easily seen.

The cortex, which is developed from the endodermis or pericycle, and is, botanically, a phelloderm or secondary cortex, consists of parenchymatous cells with rather thick walls and large pits; the walls have been swollen a little by the action of the caustic alkali, especially if the preparation has been warmed. There are numerous intercellular spaces. Near the cork the cells exhibit marked tangential elongation; towards the cambium they become more nearly isodiametric.

In the bast ring, which is not sharply delimited towards the outside, observe radially arranged groups of sieve tissue; they may be recognised by the very small diameter of the cells, their thinner walls, and the absence of pits; sieve plates may be detected by the corallin-soda reaction. There are no bast fibres.

The wood within the cambium consists largely of thin-walled parenchyma, throughout which radially elongated groups of vessels are distributed. The centre of the root is occupied by the primary wood.

The bundles contain a few (often 3 to 8) vessels, accompanied by smaller elements the nature of which is not so evident (compare radial section).

Most of the parenchymatous cells contain starch grains, but here and there a cell can be seen filled with a dark granular, often almost black, substance. Closer examination shows this to be sandy crystals, and the usual tests will indicate it to be calcium oxalate (compare Dulcamara, p. 86).

Examine the starch in water. Most of the grains are compound, and consist of two or three component granules which vary considerably in size. The majority are about 15 μ in

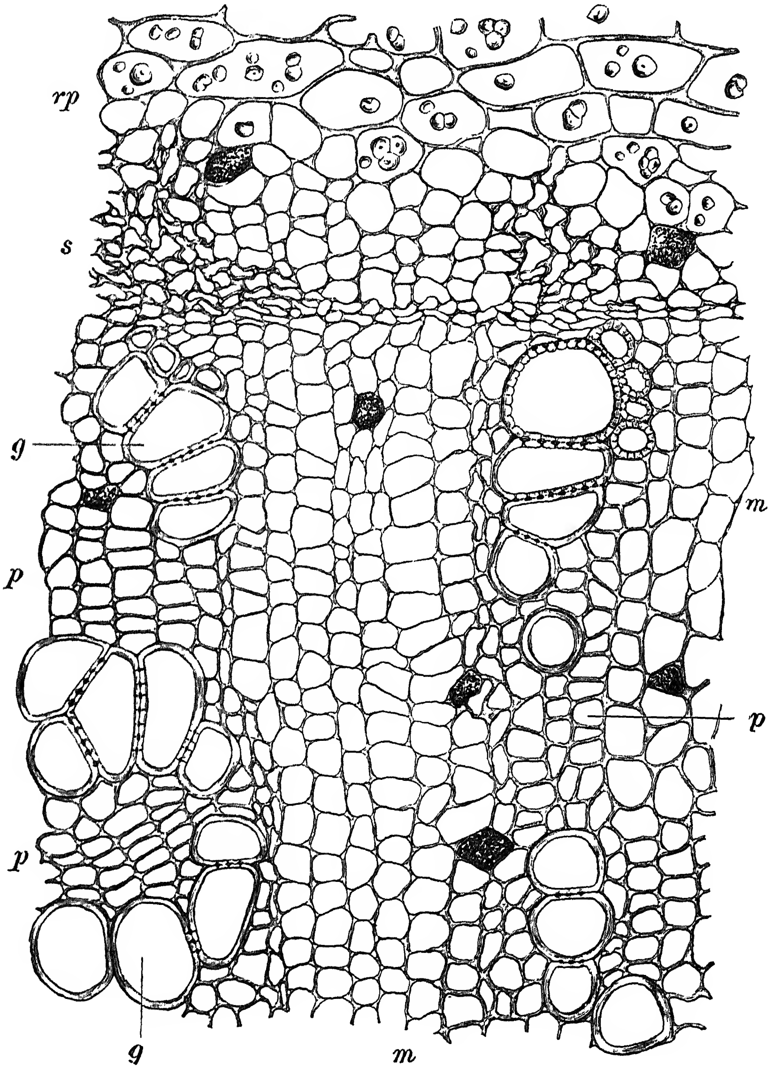

FIG. 173.—Belladonna Root, transverse section. *g*, vessels; *m*, medullary rays; *p*, parenchyma of wood; *rp*, cortical parenchyma; *s*, bast groups, with sieve tubes; the dark contents in some of the cells are sandy crystals of calcium oxalate. (Moeller.)

diameter, but some are minute, while others may attain exceptionally as much as 30 μ.

Prepare now radial sections. Most of the parenchymatous cells are axially elongated. The vessels have pitted walls. The smaller elements associated with the vessels are mostly tracheids passing by a series of intermediate forms into fibres, of which, however, but few are typical. Most of the tracheids are elongated, narrow, with bluntly pointed extremities and numerous large rounded or oval pits, often arranged in a left ascending spiral. The latter form of tracheid is intermediate between the typical tracheid and the wood fibre ; in the wood fibres the pores are slits and are by no means numerous. The cells filled with sandy crystals are conspicuous and are axially elongated.

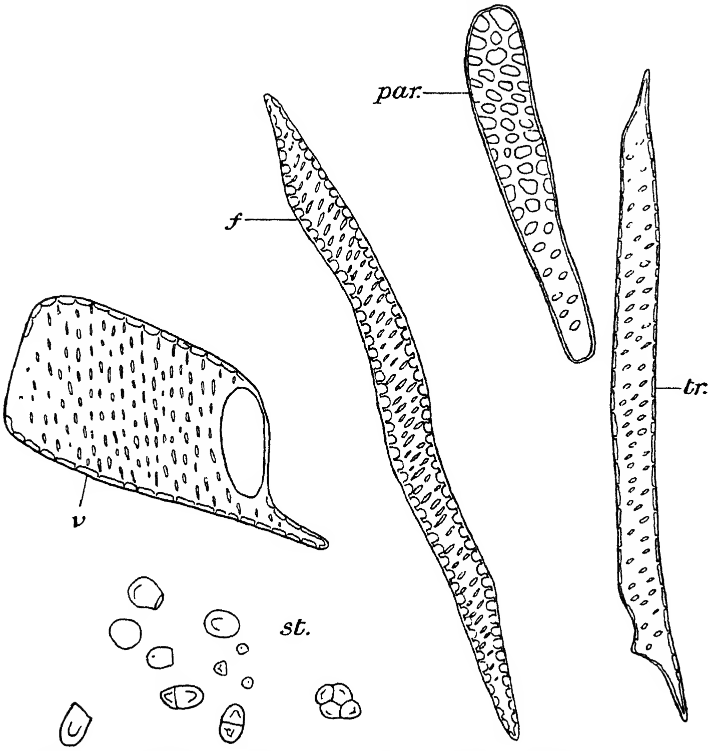

FIG. 174.—Belladonna Root, elements of wood, separated by potassium chlorate and nitric acid. *f*, fibre ; *tr.*, tracheid ; *par.*, wood parenchyma cell, reticulated at one extremity, pitted at the other ; *v*, vessel ; *st.*, starch (in water). ×200.

Marshmallow Root

Source.—The root of *Althæa officinalis,* Linn.

Examine this in the same way as belladonna. Observe especially in the cortical portion, but also in the wood, bast fibres with small lumen and rounded or polygonal outline. The starch grains are about the same size as those of belladonna

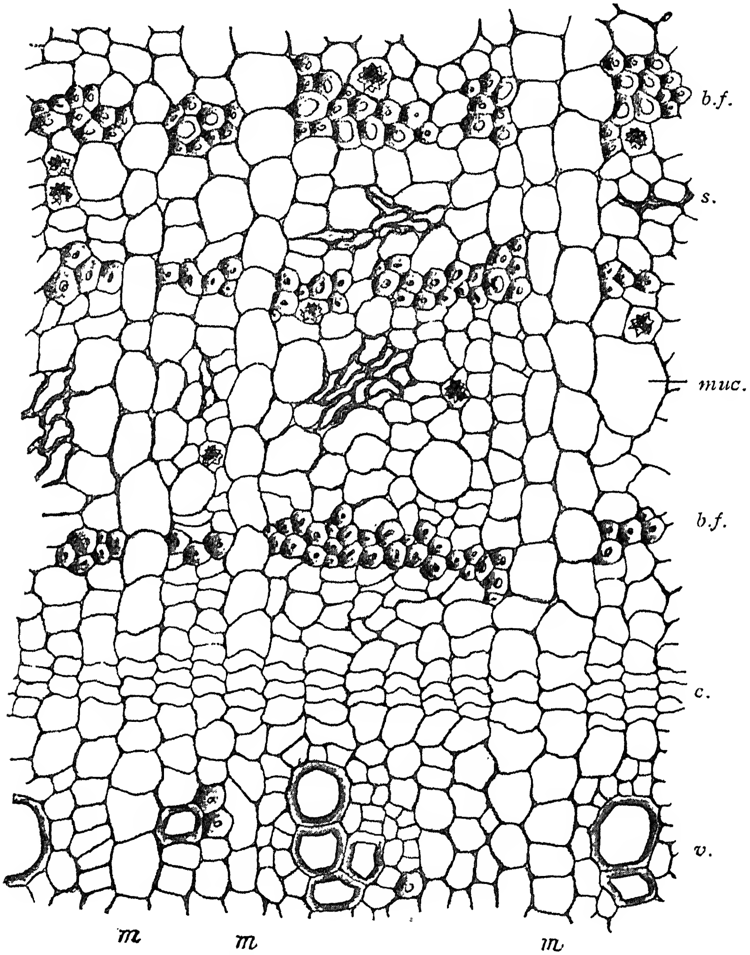

FIG. 175.—Marshmallow Root, transverse section. *b.f.*, bast fibres; *c.*, cambium; *muc.*, mucilage cell; *s.*, sieve tubes; *v.*, vessels; from a section cleared with potash. (Moeller.)

root, but they are simple, elongated, ovoid, or reniform in shape. Calcium oxalate is present in the form of cluster crystals.

In both cortex and wood there are numerous isolated parenchymatous cells filled with a colourless more or less transparent mass (mucilage). This can be stained red with ruthenium red in lead acetate. If a section is cut dry, mounted in alcohol or glycerin, and irrigated with water, the mucilage will swell, exhibit distinct stratification, and finally dissolve.

In this root the shape and length of the bast fibres, the nature of the pits in the walls, &c., should be determined by radial sections as well as by isolation.

Dandelion Root

Source.—The root of *Taraxacum officinale,* Wiggers.

Preparation and Examination.—Select for examination some small pieces of dandelion root which exhibit a pale interior when broken. Smooth the transverse surface and examine with a lens; observe the small yellowish wood and wide cortex, the latter traversed by numerous narrow, dark, concentric rings (fig. 176). Reject pieces that exhibit a pith in the centre of the wood; they are pieces of rhizome, into which the root imperceptibly passes. Expose suitable pieces to a moist atmosphere until they are ready to cut (about twelve hours). Cut transverse sections, and transfer them to alcohol.

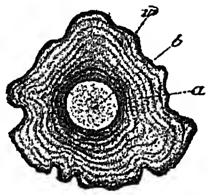

FIG. 176.–Dandelion Root, transverse section. *a,* bark; *b,* wood; *w,* cambium. ×4. (Berg.)

Examine a section in water. The wood consists of yellowish vessels irregularly intermingled with thin-walled parenchyma. The nature of the vessels can be determined, if desired, by longitudinal sections, and by isolating the elements by means of potassium chlorate and nitric acid.

Examine the cortical portion of the section. Many of the parenchymatous cells, or at least some of them, contain colourless amorphous masses of irregular shape. They consist of inulin (see later). Clear the section by gently warming (about 60 to 80° C.); the inulin dissolves completely without swelling (distinction from starch).

The cortex of the root consists of thin-walled parenchyma bounded by a thin brown line of tegumentary tissue, which, after warming with potash, is seen to consist of a few rows of cork cells with brown contents.

The concentric rings in the cortex, which are so conspicuous under a lens, are made up of a series of groups of small cells; these groups are separated from one another by a few parenchymatous cells.

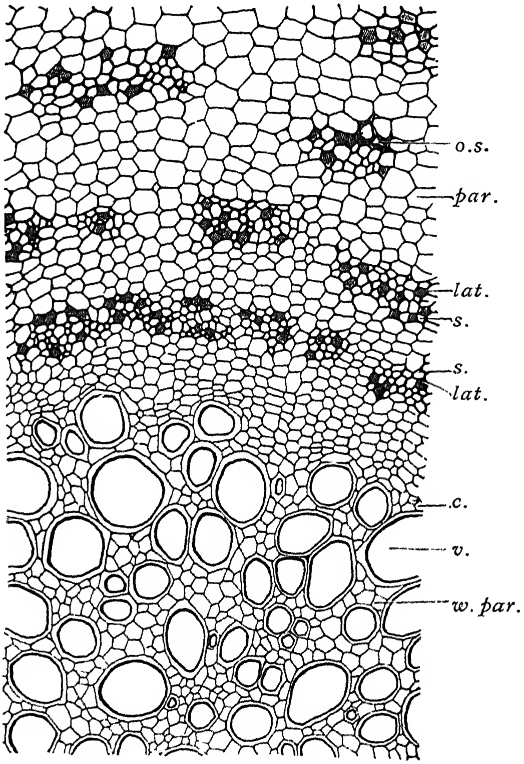

FIG. 177.—Dandelion Root, transverse section. *c.*, cambium; *lat.*, laticiferous vessels; *o.s.*, obliterated sieve tubes; *par.*, parenchyma of cortex; *s.*, sieve tubes; *v.*, vessel; *w. par.*, wood parenchyma. (Tschirch.)

Examine one of these groups closely under the high power. Some of the small cells contain a brownish or nearly black granular substance. These are laticiferous vessels.

Stain a cleared section with corallin soda. In some at least of the cells that were apparently empty a bright pink mass will be seen. This is a callus plate, and indicates that the element is a sieve tube.

Further elucidate the nature of these elements by preparing tangential sections as follows: Cut off a piece of root about 5 mm. long, and shave off longitudinally about one half of the cortex, taking care to keep the plane of the cut surface parallel to the long axis of the root; this is easily done by observing that the dark longitudinal lines (sections of the concentric rings) on the tangential section are kept parallel to one another and do not converge; then with the razor cut tangential sections until one of the concentric rings is reached and passed. Transfer these sections to water; clear as before.

The tissue consists largely of axially elongated parenchymatous cells, through which there runs an anastomosing network of laticiferous vessels with brownish granular contents; they can be seen to have lateral anastomoses with neighbouring vessels.

Stain a cleared section with corallin soda; the callus plates of the sieve tubes stain pink; the latter are numerous, and their sieve plates are often arranged in rows. With care the course of the sieve tubes may be followed; they accompany the laticiferous vessels.

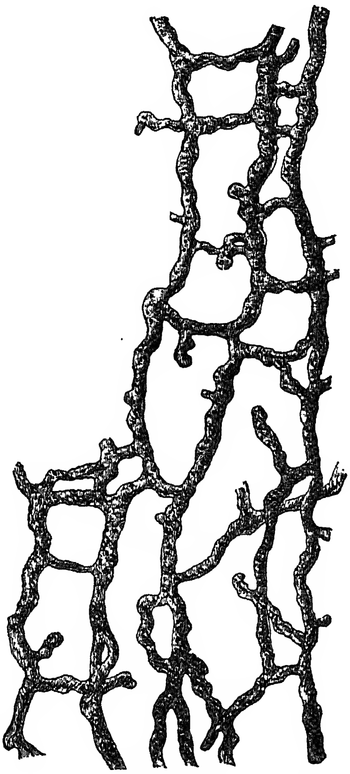

Fig. 178.—Anastomosing laticiferous vessels of Dandelion Root. ×140. (Vogl.)

Digest some tangential sections for a few minutes with solution of potash in a water-bath. The vessels can be teased out and their anastomoses easily seen.

Mount a thin tangential section in water. The chief content of the parenchymatous cells is inulin; observe it closely. The lumps are colourless, and vary very much in size and shape. Sometimes there is only one in the cell, sometimes several. Not infrequently they exhibit a more or less distinct sphærocrystalline structure; in other cases the latter is much less perceptible. Test them with iodine water; they fail to react. Warm the section very gently in water; they dissolve without swelling.

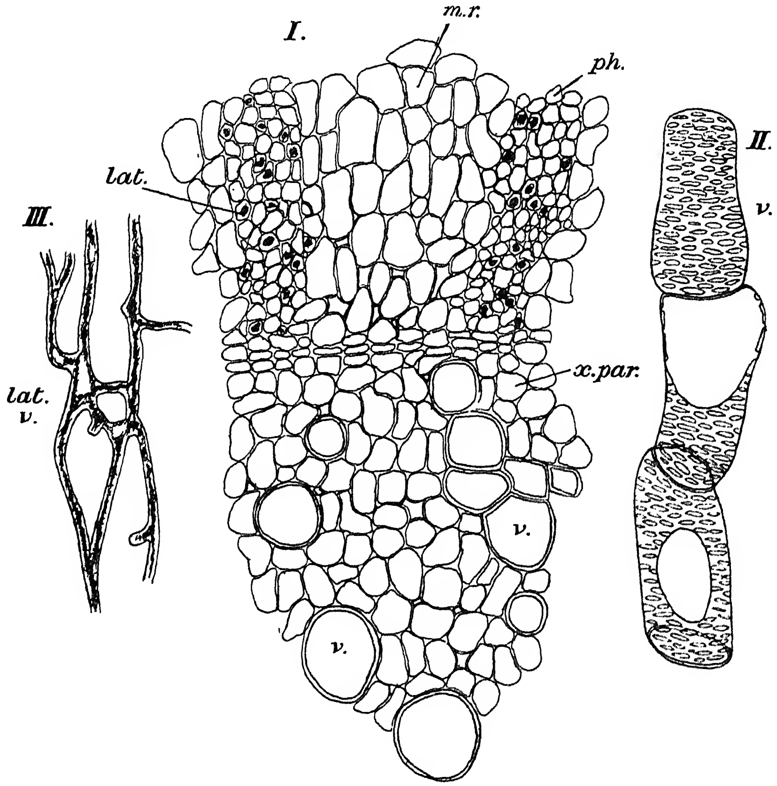

FIG. 179.—Chicory Root. I., transverse section; *lat.*, laticiferous vessel; *m.r.*, medullary ray; *ph.*, bast; *v.*, vessel; *x.par.*, wood parenchyma. II., radial section of vessels. III., laticiferous vessel isolated by maceration with potash. ×220.

Chicory

Source.—The root of *Cichorium Intybus*, Linn.

Examination.—Chicory root closely resembles dandelion root in structure, but it is usually much larger in size and not so easy to examine.

Procure from a grocer some raw chicory roots. Prepare them for cutting by soaking for fifteen minutes in water, and then hardening for three or four hours in alcohol. Cut sections with a razor flooded with alcohol, and transfer them to alcohol. Take care that they include part of the wood as well as of the cortical portion. Transfer one to a slide, mount in water, and clear with chloral hydrate.

Observe in the wood the largely developed thin-walled parenchyma in which large vessels are distributed, mostly in radial lines one or two vessels wide. The cortical portion also consists largely of parenchyma. The bast rays are narrow, and alternate with wide medullary rays. In the former groups of sieve tubes and laticiferous vessels alternate with groups of bast parenchyma.

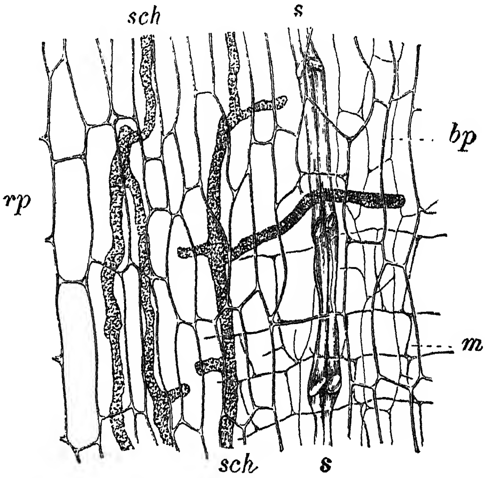

FIG. 180.—Chicory Root, radial section of cortex. *bp*, bast parenchyma; *m*, medullary ray; *rp*, cortical parenchyma; *sch*, laticiferous vessels. ×160. (Moeller.)

Examine tangential sections of the cortex; observe the anastomosing laticiferous vessels accompanied by narrow sieve tubes with small transverse plates, resembling those of the dandelion. Stain with corallin soda.

From a potash maceration preparation tease out the laticiferous vessels and examine them.

Examine also the vessels in radial or tangential sections of the wood.

Examination of Ground Roasted Chicory

Procure some ground roasted chicory; if in very coarse fragments, reduce these in a mortar to a finely granular powder.

Decolourise a portion with solution of chlorinated soda and wash. Mount a little in dilute glycerin for examination. The parenchymatous cells and the reticulate or pitted vessels are very distinct, but the laticiferous vessels are not conspicuous; they must be carefully looked for. Stain a portion of the decolourised powder with Soudan red; the laticiferous vessels are now more easily seen, as their contents stain, more or less satisfactorily, red. Stain another preparation with tincture of alkanna diluted with an equal volume of water, allowing twenty-four hours for the absorption of the stain. This method sometimes affords better results than the Soudan red. Stain another portion with corallin soda; the callus plates stain pink, but fragments of sieve tissue staining with corallin are not very numerous in the powder.

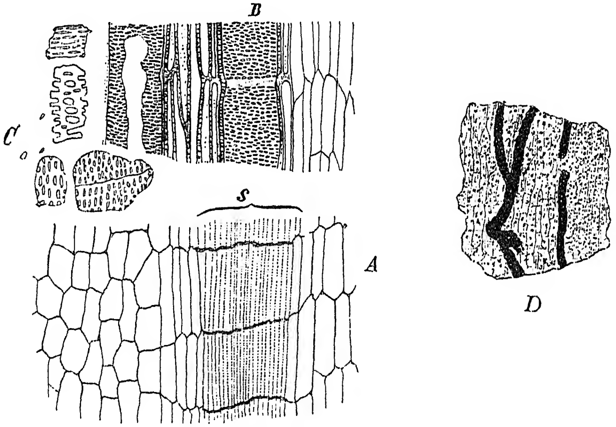

FIG. 181.—Chicory Root, fragments from the powder. *A*, portion of the cortex in longitudinal section, showing the sieve tubes, *s*, with sieve plates. *B*, portion of the wood in longitudinal section, showing the vessels; ×70. *C*, portions of the walls of the vessels. ×240. *D*, fragment of parenchyma of cortex with laticiferous vessels. ×120. (Schimper.)

Another portion of the ground chicory may be digested with solution of potash in a water-bath for fifteen minutes, washed, and examined in glycerin or chloral hydrate. This preparation often affords good results.

The following are the diagnostic characters of chicory root:

(*a*) Abundant parenchymatous tissue in wood and cortex.

(*b*) In the cortex, laticiferous vessels and numerous small sieve tubes with transverse plates.

(*c*) In the wood, vessels of considerable size with large pits.

Ipecacuanha Root

Source.—The root of *Psychotria Ipecacuanha*, Stokes.

Preparation and Examination.—Select several typical pieces of Brazilian ipecacuanha root, and soak them in water or dilute glycerin until sufficiently soft to cut (twenty-four hours or more). Cut transverse sections, and treat in the usual way.

Examine one in water; it contains abundance of starch; remove this by clearing.

The tegumentary tissue consists of several rows of thin-walled flattened cells containing a brown granular substance, to which the dark-brown colour of the root is due; in surface view these cells exhibit an irregular polygonal outline.

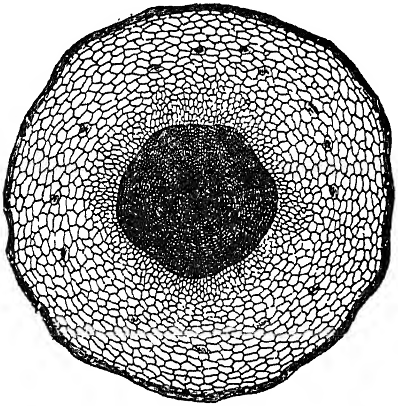

Fig. 182.—Ipecacuanha Root, transverse section, showing dense wood and large cortex. Magnified. (Planchon and Collin.)

The parenchymatous tissue that follows the cork is secondary cortex and consists of rounded or polygonal cells that are often axially elongated and exhibit few intercellular spaces. It passes, without any visible line of demarcation, into the bast ring, the bast itself forming wedge-shaped groups of cells, recognisable by their smaller size and by the difference in the cell walls. Here and there in the secondary cortex and bast are cells filled with what often appears to be a granular substance, but which, on closer examination, especially of radial sections, proves to be acicular crystals of calcium oxalate.

The wood consists almost entirely of small elements, vessels not being distinguishable. The medullary rays are not conspicuous, all the cells of the wood showing, in transverse section, about the same radial elongation.

Stain a section, before clearing, with solution of iodine; more or less regular radial lines of cells, containing abundance of starch, can be detected. These cells probably correspond to medullary rays and perform the function of such. A tangential section of the wood shows that they are strongly axially elongated and bear simple pits, but otherwise are not strikingly different from the other elements of the wood.

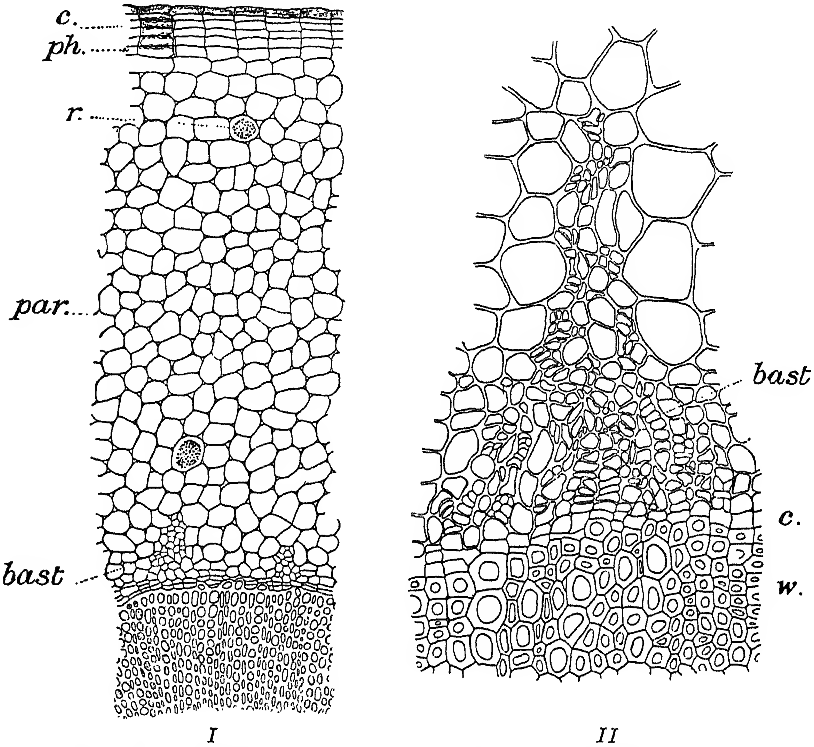

FIG. 183.—Ipecacuanha Root. I, transverse section of the cortex, with part of the wood; *c*, cork; *par.*, parenchyma of cortex; *ph.*, phellogen; *r.*, raphides. II, portion of the same, more highly magnified; *c.*, cambium; *w.*, wood. (After Tschirch.)

Macerate some fragments of the wood with potassium chlorate and nitric acid; examine the cells of which it consists. Vessels, at least typical vessels, are not to be found, but there are numerous tracheids and fibrous cells. The tracheids (fig. 184, *tr.*) have more or less pointed ends and oblique transverse walls, in which a perforation may generally be detected. The pores are commonly oval; they ascend in a left spiral, and are bordered. The fibrous cells have slit pores also arranged in a left spiral. They differ from ordinary wood fibres chiefly in the fact that they contain starch, and hence are not solely mechanical in their function. In addition to these two forms of cells, typical wood parenchyma (fig. 184, *par.*) may be found, as well as cells that are intermediate in character. True vessels are absent.

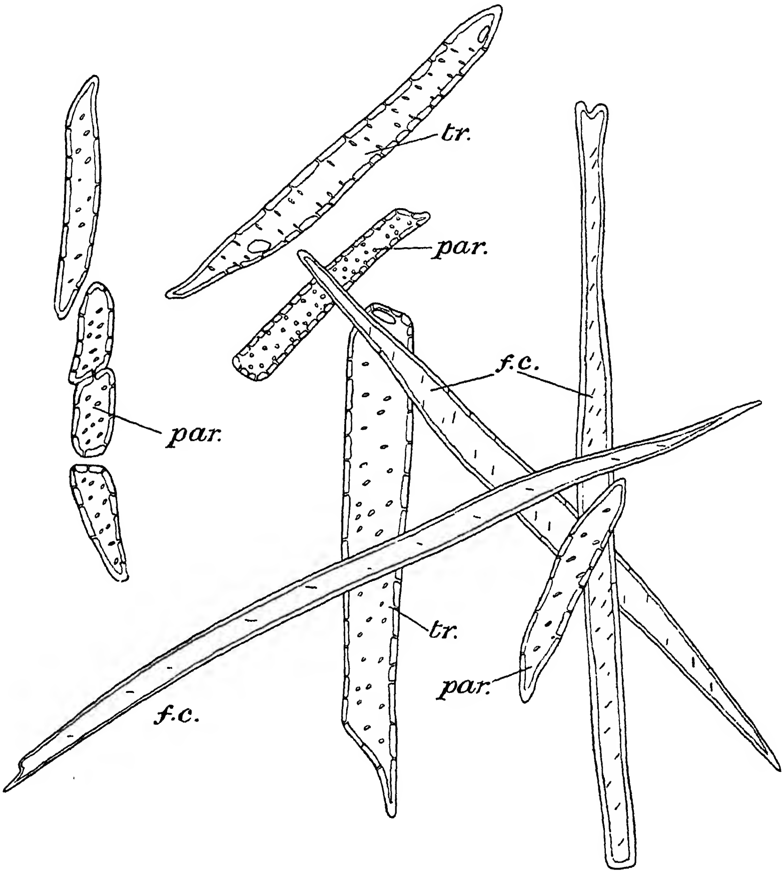

Fig. 184.—Brazilian Ipecacuanha, elements of root wood. *f.c.*, fibrous cells; *par.*, parenchymatous cells; *tr.*, perforated tracheids. ×250.

Examine next the starch grains; these can easily be separated by scraping the cut surface of a dry root. They are mostly compound. The component grains number commonly from two to four, occasionally as many as eight or even more, and are most frequently muller-shaped, with one or two flat surfaces. The hilum is usually a distinct point, or its position may be indicated by a simple or triangular cleft. In length the grains occasionally reach 12·5 μ, but never exceed 15 μ.

Proceed next to examine the powdered drug. Mount a little in water or dilute glycerin, and examine the starch grains.

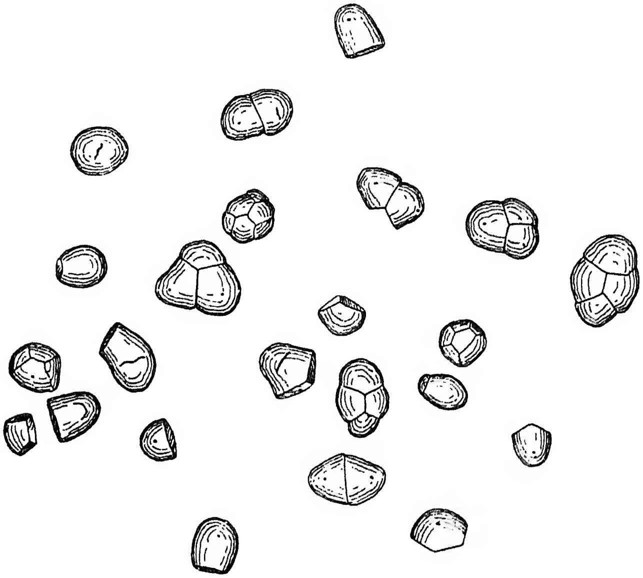

FIG. 185.—Starch of Brazilian Ipecacuanha Root. ×750.

Note the presence here and there of an acicular crystal of calcium oxalate and of fragments of parenchymatous cells, as well as of wood. The details of the latter cannot well be identified without further preparation.

Mix about 0·5 gramme of the powder with 5 c.c. of water, and raise the mixture to the boiling-point, so as to gelatinise the starch; add 10 c.c. of nitric acid (sp. gr. 1·42) and about 0·5 gramme of potassium chlorate. Warm gently for about five minutes, taking care that the action is not too vigorous. Dilute with about an equal volume of water, and separate by the centrifuge the tissues that have resisted destruction ; wash once with water, separating by the centrifuge as before ; then treat with a few cubic centimetres of chloral hydrate for a few minutes, and separate again. Pour off the chloral hydrate solution, and examine the residue either in water, dilute glycerin, or chloral hydrate. No elements, especially no

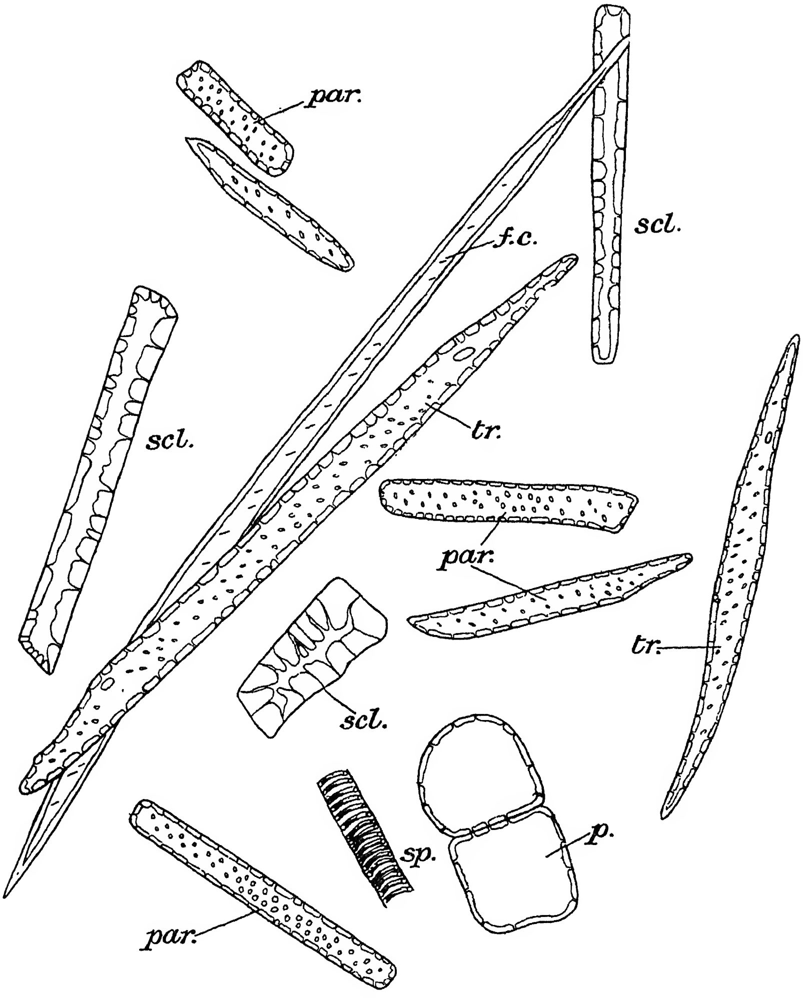

FIG. 186.—Brazilian Ipecacuanha, elements of stem wood. *f.c.*, fibrous cells ; *p.*, lignified cells of pith ; *par.*, parenchymatous cells ; *scl.*, sclerenchymatous cells ; *sp.*, spiral vessel. ×250.

sclerenchymatous elements, but those previously found in the root should be present. The characteristic tracheids of the wood can be well seen and identified, but the perforations in the oblique walls are sometimes difficult to find. Parenchyma that has escaped destruction is fairly abundant, and numerous fragments of cork may be found. The tracheids may be separated from one another by gently pressing upon and at the same time sliding the coverslip.

Ipecacuanha powder frequently contains a considerable proportion of the erect stem; this betrays itself (1) by the sclerenchymatous cells of the pericycle, (2) by the lignified cells of the pith, and (3) by the spiral vessels of the protoxylem.

Cartagena ipecacuanha, which is sometimes substituted for the Brazilian, is chiefly to be distinguished by its starch grains, which are, as a rule, larger than those of the Brazilian root. Large grains frequently attain 17 to 22 μ, the latter figure being rarely exceeded, whereas, in the Brazilian root, 15 μ is the maximum. It must, however, be observed that the size of the starch grains is somewhat variable, the maximum size being attained in the largest roots; hence, small roots of Cartagena ipecacuanha and large roots of the Brazilian drug contain starch grains of approximately the same size, and the powders derived from such roots cannot, therefore, be distinguished by this means.

The powder of any root that contains vessels in its wood can be easily detected by the presence of these in the sclerenchymatous tissue separated by treatment with potassium chlorate and nitric acid.

Liquorice Root

Source.—The dried root of *Glycyrrhiza glabra*, Linn.

Preparation and Examination of Sections.—Select a thin, unpeeled Spanish liquorice root, cut off a piece about 2 cm. long, split it longitudinally into four, and soak the fragments for a week or more in dilute glycerin. Embed one in pith and cut transverse sections, taking care that some at least shall extend from the cork to within the cambium; transfer them to dilute glycerin or to water. Mount one or two in dilute glycerin.

Examine with the low power. Observe the reddish-brown cork, the groups of bast fibres in the secondary bast, the large vessels with thick yellow walls, and the groups of sclerenchymatous fibres in the wood. The parenchymatous cells constituting most of the remaining tissue are packed with small starch grains. The cambium is usually inconspicuous. Near it, in the secondary bast, note the large groups of sieve tissue, recognisable by the small irregular cells with wavy walls. Between these and the cork, strands of collapsed sieve tissue (fig. 187) can be detected.

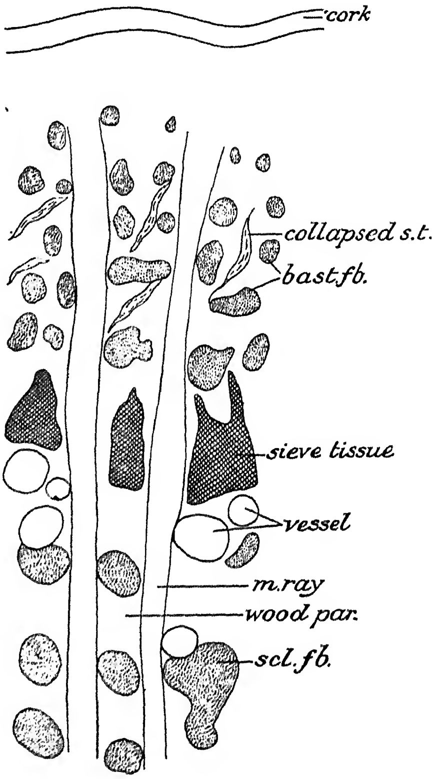

FIG. 187.—Liquorice Root. Diagrammatic transverse section. ×40.

Examine with the high power. The cork consists of numerous (10 to 20 or more) rows of narrow cells. The outer are filled with a reddish-brown, amorphous substance, the inner three or four rows have rather thicker, colourless walls and are empty;

abutting on the phellogen are usually one or two layers of phelloderm in which single isolated crystals of calcium oxalate may be found. Note the thin walls of the parenchymatous cells. Further details are best examined after clearing.

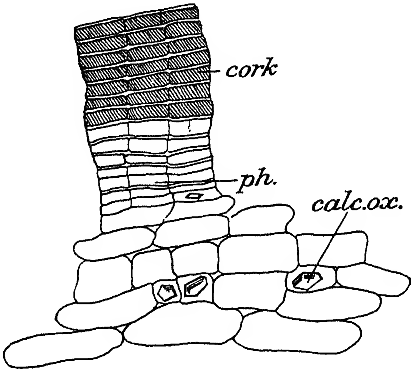

FIG. 188.—Liquorice Root, transverse section of cork with portion of cortex. *ph.*, phellogen; *calc. ox.*, calcium oxalate. ×240.

Clear a section by gently warming with solution of chloral hydrate. Observe in the cortex isolated crystals of calcium oxalate in small cells. The colouring matter in the cork cells dissolves and the cell walls can be easily seen.

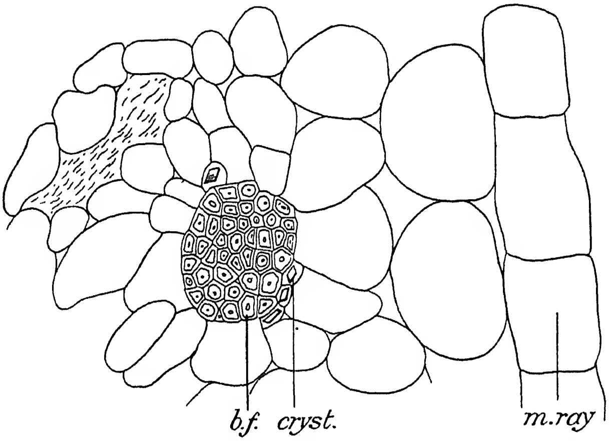

FIG. 189.—Liquorice Root, transverse section of portion of bast ring. *b.f.*, bast fibres; *cryst.*, crystals of calcium oxalate; *m. ray*, medullary ray. ×240.

Examine the bast fibres; they are much thickened, the lumen being generally reduced to a point; the middle lamella is very distinct. Abutting on the fibres are crystal cells.

Examine the vessels; note the very thick yellow wall. Each vessel is surrounded by a ring of smaller elements with thinner, yellow walls (tracheids and sclerenchymatous cells). The sclerenchymatous fibres of the wood resemble the bast fibres. The medullary rays are usually three to five cells wide. Both these cells and those of the parenchymatous tissue have thin walls.

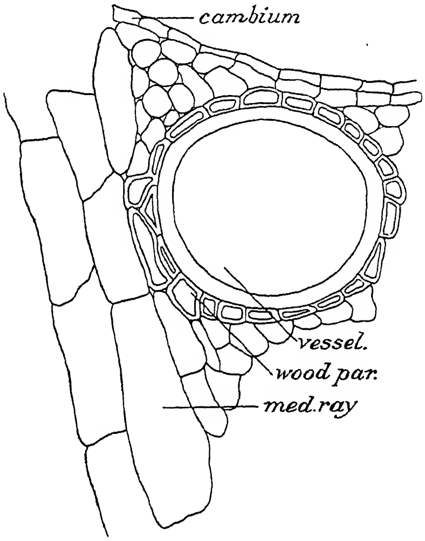

Fig. 190.—Liquorice Root, transverse section of vessel and adjacent wood parenchyma. ×240.

From one of the soaked pieces of root cut off a fragment about 5 mm. long and from it cut longitudinal sections. Clear with chloral hydrate. Examine the vessels, which are very conspicuous. Each is composed of short constituent elements; the largest bear very conspicuous, closely packed, oval, bordered pits with narrow slits; others exhibit transversely elongated oval pits or may be reticulated. In favourable portions the adjacent tracheids and thickened parenchymatous cells may be observed. Examine also the sclerenchymatous fibres. Note the absence of pits, and the presence of abundant crystal cells abutting on the fibres. Examine one or two of the crystals where they are best seen; each crystal is surrounded by a thickened, lignified wall (phloroglucin reaction). The walls of the vessels, tracheids and thickened parenchymatous cells are also strongly lignified, but in the groups of sclerenchymatous fibres it is only the middle lamella which is strongly lignified.

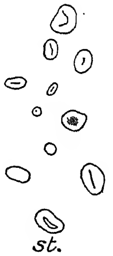

FIG. 191.—Liquorice Root, starch. ×240.

From a portion of the dry root scrape a little powder, mount in water, and examine the starch. The grains are single (very seldom compound), rounded, oval or irregularly four-sided; in the centre is a punctiform hilum or an elongated linear or oval cleft; the grains reach 18 μ in length, but the majority vary from 3 to 10 μ.

Examination of Powdered Liquorice Root.—Mix a little of the fine powder of commerce with (*a*) water; (*b*) dilute glycerin; and (*c*) solution of chloral hydrate. The first may be examined after standing an hour, but (*b*) and (*c*) should be allowed to stand from twelve to twenty-four hours.

Mount a little of (*a*) in water, taking care not to take too much of the material. Examine under the low power and note the yellowish colour of the larger fragments (vessels and sclerenchymatous fibres). Examine with the high power. Observe the abundant starch grains; in many samples of commercial powdered liquorice root a number will appear gelatinised in the centre or partially disintegrated (the result of overheating). Observe also numberless minute granules which stain yellow with solution of iodine in potassium iodide; they are protoplasmic granules. Crystals of calcium oxalate may also be found; they are fairly numerous; but many are broken, and then can best be detected by the use of the polariser. Flat, irregular, almost transparent pieces of the walls of parenchymatous cells are abundant; most of them exhibit their surfaces, but some show their sections, and it will then be seen that they are thin. Fragments exhibiting both surfaces and sections may readily be found as well as the junction of two or more cells. Large groups may also be found, the cells of which are still filled with starch grains.

In addition to the above, small and large fragments of vessels are frequent; they are yellowish in colour and exhibit pits, but they are better examined in the chloral hydrate preparation.

The sclerenchymatous fibres are also readily found. The groups, which are usually broken transversely, are yellowish in colour and are generally accompanied by crystals of calcium oxalate; some of the fragments are more or less distorted and disintegrated by the grinding, and numerous portions of separated fibres may be found; these are also best examined in the chloral hydrate preparation.

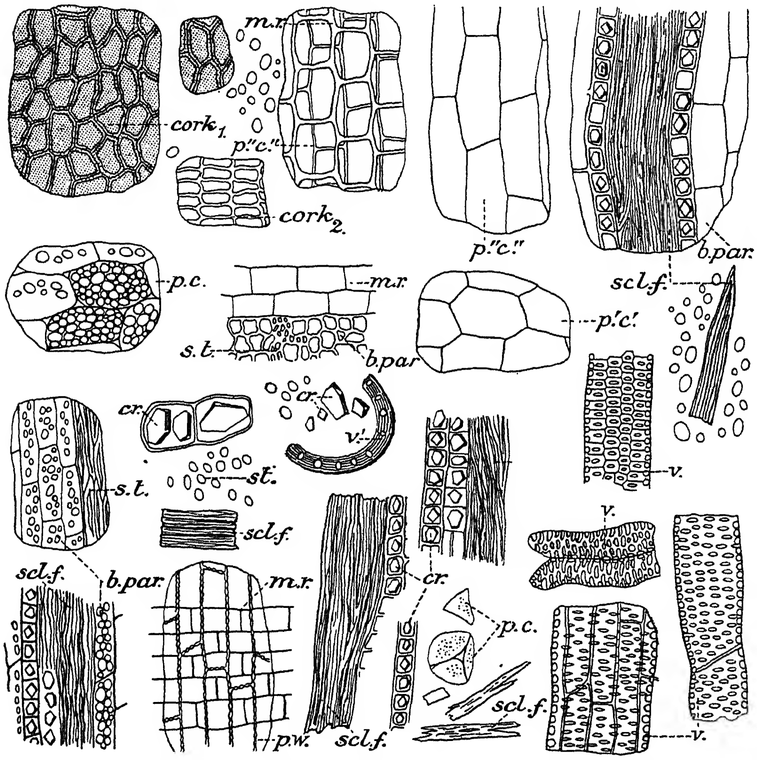

FIG. 192.—Powdered Liquorice Root. *b.par.*, bast parenchyma; *cork*$_1$, *cork*$_2$, cork in surface view and in section; *cr.*, crystals of calcium oxalate; *m.r.*, medullary ray; *p.c.*, fragments of cells of cortical parenchyma; *p.'c.'*, *p."c."*, cortical parenchyma in transverse and in longitudinal section; *p.w.*, parenchyma of wood; *scl.f.*, sclerenchymatous fibres; *s.t.*, sieve tissue; *st.*, starch; *v.*, vessel. (Partly after Greenish and Collin.) ×150.

Mount a little of (*b*) in dilute glycerin and examine.

The fragments of tissue are clearer, but the starch grains do not show their details so well. Examine more especially the larger groups of parenchymatous cells.

In preparation (*c*) the starch grains will have been gelatinised and many of the protoplasmic granules dissolved. The characteristic pits on the vessels can be readily seen. The groups of bast fibres with their accompanying crystals are very conspicuous; occasionally portions of a single fibre may be seen, while shreds and fragments of fibres that have been disintegrated by the grinding are frequent. In favourable instances the thickened membrane enclosing the calcium oxalate crystal can be discerned. The groups of parenchymatous cells, now freed from starch, are quite clear; fragments of these groups of various sizes are abundant and are more distinctly seen than in (*a*), but the pits are less readily visible. The large rounded cells of the parenchyma of bast and wood, with intercellular spaces, can be distinguished from the radially elongated cells of the medullary rays.

In none of the preparations are fragments of cork to be found, as the powder is prepared from decorticated root.

Gentian Root

Source.—The dried root of *Gentiana lutea*, Linn.

Preparation and Examination of Sections.—Select three or four gentian roots about 1 cm. in diameter and cut from each a piece about 2 cm. long. Soak these in distilled water for two days, then transfer them to 45 per cent. alcohol for twenty-four hours, and finally to 75 per cent. alcohol, in which they can remain till required. Air-dry gentian root, or root that has been exposed to a moist atmosphere for a day or two, is in a very suitable condition for section-cutting, but the parenchymatous cells are much collapsed and do not readily resume their normal shape when the sections are transferred to water, thus rendering the structure difficult to understand.

Cut transverse sections, using a razor moistened with the alcohol; take care to include the cork, the bast ring and part of the wood. Transfer them to 70 per cent. alcohol. Mount one in dilute glycerin and examine under the low power.

On the outside is a narrow layer of cork the individual cells of which are not very distinct.

Mount another section in solution of chloral hydrate, warm gently and examine first with the low and then with the high power. The cells are large, strongly tangentially elongated and thin-walled; they contain globules of oil with an occasional small crystal of calcium oxalate.

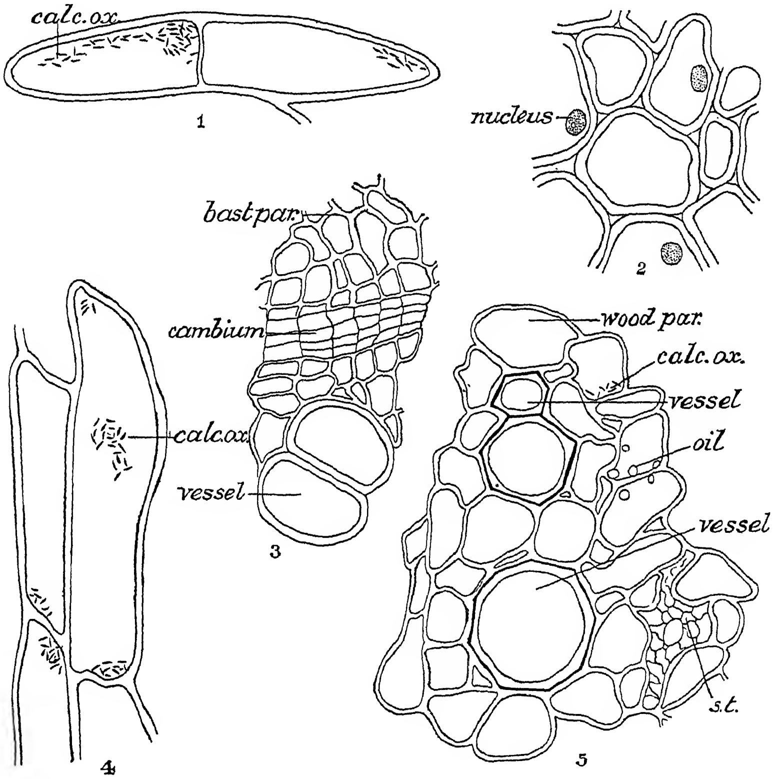

FIG. 193.—Gentian Root. 1, transverse section of cortical cells (near the cork) with crystals of calcium oxalate; 2, of outer portion of bast ring, showing distinct nucleus; 3, of cambium with adjacent bast parenchyma and wood; 4, radial section of parenchyma; 5, transverse section of portion of wood with sieve tissue, *s.t.* All after prolonged soaking in dilute glycerin; in 5 much of the calcium oxalate and oil omitted. ×240.

Proceed with the examination of the dilute glycerin preparation. The narrow cortex consists of strongly tangentially elongated thick-walled cells (fig. 193, 1), containing a granular substance (to be examined later). The bast ring is wide. As the middle portion is approached the cells become rather larger, more rounded, the walls thinner, and intercellular spaces conspicuous (fig. 193, 2); towards the cambium the cells and the intercellular spaces diminish in size (fig. 193, 3). Groups of small-celled sieve tissue are scattered throughout the bast ring, but they are inconspicuous and require careful search. Neither sclerenchymatous cells nor fibres are present, nor can medullary rays or bast rays be distinguished.

Within the cambium is the wood, which consists chiefly of parenchymatous tissue resembling that of the bast ring and containing similar groups of sieve tissue; the vessels are large (50 to 100 μ) and either scattered or in groups exhibiting, especially near the cambium, a more or less distinct radial arrangement (fig. 193, 5).

The contents of the parenchymatous cells should be examined in sections prepared from a drug that has not been subjected to prolonged treatment with water and alcohol. From an air-dry root, or one that has been kept for a day or two in a moist atmosphere, cut transverse sections. Mount one in dilute glycerin, warming gently, if necessary, to expel air. Examine the cell contents. Small globules of oil are more or less abundant; they may be stained by immersion for half an hour in diluted tincture of alkanna. Many of the cells contain numerous granules which stain with solution of iodine in potassium iodide; they are protoplasmic granules.

For further examination of the cell contents defat some sections in a small tube or dish with ether-alcohol, transfer to alcohol, mount in water, and replace the water by solution of chloral hydrate. Examine under the high power. In some of the parenchymatous cells minute prismatic crystals of calcium oxalate will be found. These crystals vary much in quantity and distribution, being sometimes present in great numbers and in many cells, sometimes in small numbers and in few cells. They may often be found in the cells of the cortex near the cork. They are very characteristic.

Next cut radial sections from the air-dry root; clear with chloral hydrate as above described. Examine the vessels; they bear large transversely elongated pits or are reticulately thickened. Examine also the parenchymatous tissue for calcium oxalate, which is usually more conspicuous in a radial than in a transverse section (fig. 193, 4). Occasionally a strand of sieve tissue may be found in the radial section.

Expand a defatted section in water and replace the water by chloral iodine. Observe the absence or almost total absence of starch.

Examine a defatted section in dilute glycerin; note the large cell-nucleus which is often conspicuous.

Examination of the Powder.—Mix a little powder (*a*) with water and (*b*) with dilute glycerin and set them aside for several hours. Defat a little by shaking it with ether-alcohol; pour off the ether-alcohol, transfer the powder to a small dish and allow it to dry. Mount a little in solution of chloral hydrate (*c*).

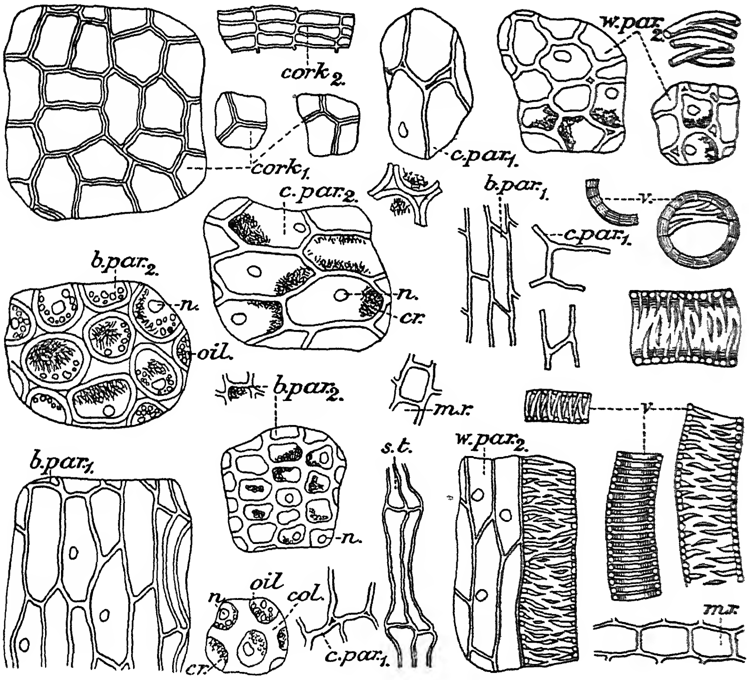

FIG. 194.—Powdered Gentian Root. *b.par*$_1$., *b.par*$_2$., bast parenchyma in longitudinal and in transverse section; *c.par*$_1$., *c.par*$_2$., cortical parenchyma, in longitudinal and in transverse section; *col.*, collenchymatous cells; *cork*$_1$, *cork*$_2$, cork in surface view and in section; *cr.*, crystals of calcium oxalate; *m.r.*, medullary ray; *n.*, nucleus; *oil*, oily globules; *s.t.*, sieve tubes; *w.par.*, wood parenchyma; *v.*, vessel. After Greenish and Collin. ×150.

Examine (*a*) first. Note the numerous minute protoplasmic granules similar to those seen in the transverse sections. Oil globules are scarcely to be found even after treatment with tincture of alkanna, the oil having apparently been distributed over the tissue by the grinding. Observe in the water preparation numerous small, irregular, transparent fragments of the cell walls showing usually their surface but sometimes their sections ; in the latter case the thickness of the wall should be noted and compared with the walls in the entire drug. Larger fragments showing two or three adjoining cells with an intercellular space may also be seen. Note also the colour which is pale yellowish-brown in the larger pieces ; small fragments are colourless or nearly so.

Examine next the dilute glycerin preparation (*b*). It is clearer. The outlines of the cells in the larger fragments can be distinguished. Observe the great variety; they are mostly elongated, but the walls vary much in thickness and, while some cells are more or less collapsed, others are expanded ; many show intercellular spaces. Fragments of the vessels with the characteristic reticulated, annular or occasionally pitted walls may also be seen.

Examine lastly the chloral hydrate preparation (*c*). In this the larger fragments are still clearer ; the characteristic calcium oxalate crystals are more easily found in this preparation than in either of the preceding, and the walls of the vessels are better cleared for examination.

Note particularly in the powder the absence of starch grains and of any sclerenchymatous cells or fibres.

Fragments of the cork showing usually the surface view are to be found, but they require diligent search.

The chief diagnostic characters of powdered gentian are :

(*a*) The abundant, rather thick-walled parenchymatous cells often containing minute crystals of calcium oxalate and a little oil, but no starch.
(*b*) The absence of sclerenchymatous cells or fibres.
(*c*) The large reticulated, scalariform or annular vessels.

Calumba Root

Source.—The sliced, dried root of *Jateorhiza Calumba*, Miers.

Preparation and Examination of Sections.—Cut a slice of calumba root radially into five or six pieces ; expose these for several days to a moist atmosphere. Smooth the transverse surface of one of them and note the position of the cambium.

Cut a piece about 5 mm. wide and 10 mm. high, embracing the cork and the cambium. Embed in pith, cut transverse sections and keep them in a small dish.

Transfer one or two to a slide ; moisten with alcohol, mount, after a minute or two, in water and examine. The tissue consists almost entirely of large parenchymatous cells filled with starch. On the outer side there is dark brownish-yellow layer of cork, the details of which are not readily seen ; on the inner side the cambium and a small portion of the wood may be visible.

Mount a section in solution of chloral hydrate and warm gently. The cork expands ; there are numerous rows of typical thin-walled cork cells which, in some roots, are interrupted by bands of cells of less regular shape. The parenchymatous tissue consists of large thin-walled cells, tangentially elongated near the cork, but becoming radially elongated in the bast ring. Traversing the parenchyma from the cambium to near the cork are narrow lines of sieve tissue, which is collapsed, except in the portion abutting on the cambium. Close under the cork are sclerenchymatous cells conspicuous by reason of their bright yellow walls. Examine these carefully. The walls are usually irregularly thickened, one of them being thinner than the others or even not thickened at all ; they are coarsely pitted. In the cell cavity prismatic crystals of calcium oxalate are usually visible and are often conspicuous ; they are well defined, sometimes large, two or three completely filling the cell, sometimes small. The distribution of these cells is irregular ; in some sections but one or two may be visible, in others several may occur close together. Large nodules of calcium oxalate occasionally occur just below the cork. No sclerenchymatous fibres are to be found. From the transverse surface scrape a little powder, mount in water and examine the starch. The grains vary much in size, the large from 20 to 70 μ and the small from 8 to 20 μ ; they are mostly simple, and irregularly ovoid, pear-shaped or rounded with a conspicuous cleft or stellate hilum. Compound grains with from two to six small component grains may be found.

Cut now from the root a piece that shall include the cambium and part of the wood. Treat as before. Here too the tissue consists chiefly of large, thin-walled, parenchymatous cells packed with starch grains. The vessels are large, with moderately thick yellow walls, and are arranged in radial groups and lines; they are accompanied by small vessels, interspersed with which are cells with thin walls exhibiting at most but slight lignification.

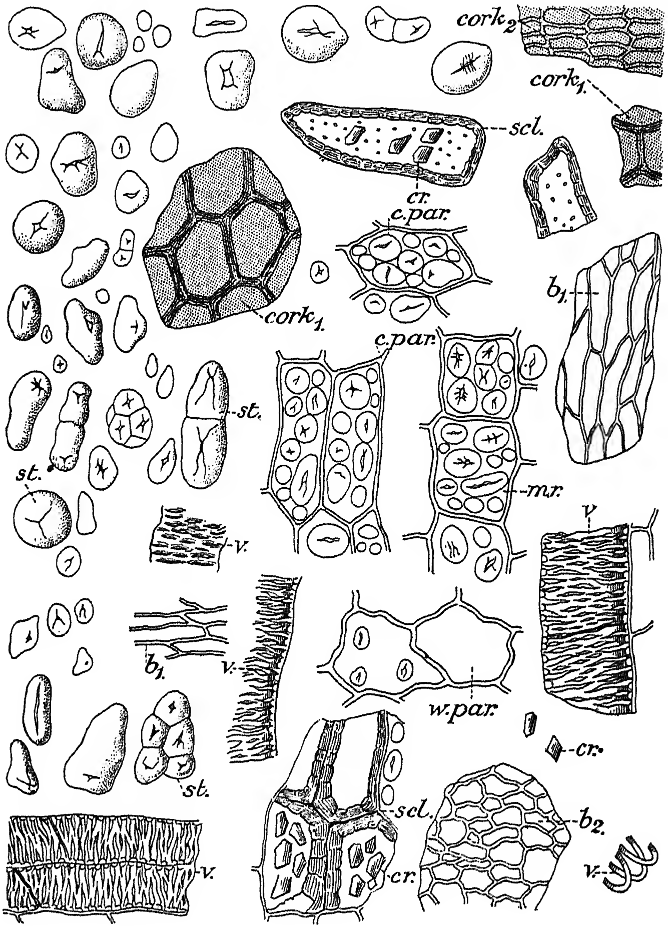

FIG. 195.—Powdered Calumba Root. b_1., b_2., bast in longitudinal and transverse section; *c.par.*, cortical parenchyma; *cork*$_1$, *cork*$_2$, cork in surface view and in section; *cr.*, crystals of calcium oxalate; *m.r.*, medullary ray; *scl.*, sclerenchymatous cells; *st.*, starch; *v.*, vessel; *w.par.*, wood parenchyma. (After Greenish and Collin.) ×150.

Cut also radial or tangential sections through the wood near the cambium. Clear with chloral hydrate and examine the vessels. The walls are reticulately thickened or exhibit transversely elongated or occasionally oval pits.

Examination of Powdered Calumba.—Mix a little finely powdered calumba root with (*a*) water, and (*b*) chloral hydrate; allow them to stand in well-covered watch-glasses for several hours.

Examine (*a*) first. Conspicuous everywhere are the starch grains which, in commercial powders, often show traces of overheating. Large and small portions of the walls of the parenchymatous cells are abundant; most of them exhibit their surfaces, but some show their profiles and allow of the thickness of the wall being noted; here and there fragments including the junction of two or more cells may be found. Granules of protoplasm are abundant and occasionally a crystal of calcium oxalate may be met with. Sclerenchymatous cells, broken or entire, in the latter case containing calcium oxalate crystals, although not abundant, are readily detected by their bright yellow colour. Fragments of the vessels, not quite so bright in colour, are frequent, and so are also groups of vessels. Portions of the cork may be recognised by their brownish colour, but the cells are not very readily distinguished.

Examine (*b*). If the preparation has stood long enough, the starch will be gelatinised, otherwise this can be effected by gently warming. The sclerenchymatous cells and the vessels have lost much of their colour, but are clearer; the calcium oxalate crystals are more distinct. The cells of the cork have expanded, and portions exhibiting the section as well as the surface may be found; in the latter the tiers of cells are usually distinct; they vary from yellowish to brown in colour.

The chief diagnostic characters of powdered calumba are:

(*a*) The starch grains.
(*b*) The yellow sclerenchymatous cells with calcium oxalate.
(*c*) The yellow vessels.

SECTION XIV

ADULTERANTS OF FOOD AND DRUG POWDERS

The systematic microscopical examination of commercial food and drug powders has revealed the more or less frequent recurrence of a number of less valuable substances used for the purpose of sophistication. Such substances, suitably adjusted in the fineness of their powder if simple, or in their composition if mixed, have from time to time been openly offered for sale in large quantities. They generally consist of by-products obtained in various industrial operations and are of inferior value, if not absolutely worthless, as either foods or drugs. The more important of these adulterants may be classified as follows :

(i) By-products obtained in the milling of wheat, barley, rice, oats, rye and maize ; bran, &c. ; maize cobs (cob meal).
(ii) The cake produced in the extraction of certain fixed oils : linseed, ground-nut, almond, rape, cocoa-nut, palm-nut, and others.
(iii) Shells obtained in shelling nuts : almond, walnut, cocoa-nut, hazel-nut and others.
(iv) Seeds, &c. : date stones, acorns, olive stones, chestnuts, beans, peas, &c.
(v) Various woods : pine-wood, quassia-wood, red sanders-wood, &c.
(vi) Exhausted residues : spent ginger, cinnamon, oak bark, &c.
(viii) Inferior dried fruits : pears, apples, &c.

In the following pages the attention of the student will be directed to the leading characteristics of some of these adulterants.

I.—Cereal By-Products

Apart from the valuable diagnostic features of the starch grains (figs. 8 to 11), the chief distinctive characters are the following :[1]

(*a*) *Wheat.*—The walls of the epidermal cells are straight and fairly regular. The transverse cells have straight thick walls with regular, distinct pits ; the ends of these cells are seldom rounded and there are few intercellular spaces. The cavities in the hairs are usually abruptly enlarged towards the base.

(*b*) *Barley.*—Very sharply characterised by the never-failing epidermis of the paleæ, the cells of which have very sinuous walls and are interrupted by crescent-shaped single or twin cells or circular cells sometimes elongated into very short conical hairs. Below the epidermis is a layer of fibrous cells. The hairs are thin-walled and conical. The transverse cells have abundant intercellular spaces. The aleurone layer is two or three cells thick.

(*c*) *Rice.*—The by-product obtained in polishing husked rice is used as a source of rice starch, but it is also met with as an adulterant. In addition to the starch, it contains the thin pericarp characterised by its regularly arranged cells with wavy walls. The transverse cells are loose, very long and narrow, and crossed at right angles by tubular cells.

The husks (paleæ) are often employed as packing material, and also find their way into powders. They are readily recognised by the very characteristic cells of the outer epidermis, which resemble those of barley but have much deeper indentations. The epidermis also bears long, conical hairs. The cells of the inner epidermis are polygonal (fig. 196).

(*d*) *Oat.*—The cells of the epidermis are elongated and have thin, delicately pitted walls ; the hairs are long, thin-walled and often in pairs. The paleæ, if present, may be identified by the elongated cells of the epidermis, with wavy walls of varying thickness; these cells are interrupted by small twin cells, one of which is crescent-shaped, or by circular cells or by scattered shortly-conical hairs (50 μ). Below the epidermis is a fibrous hypoderma followed by stellate parenchyma.

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[1] Compare Greenish and Collin, *Anatomical Atlas of Vegetable Powders.*

(*e*) *Rye.*—The epidermal cells resemble those of wheat, but are thinner and less regularly pitted. The ends of the transverse cells are mostly rounded, leaving distinct intercellular spaces; the walls are scarcely pitted. The cavities in the hairs usually taper gradually.

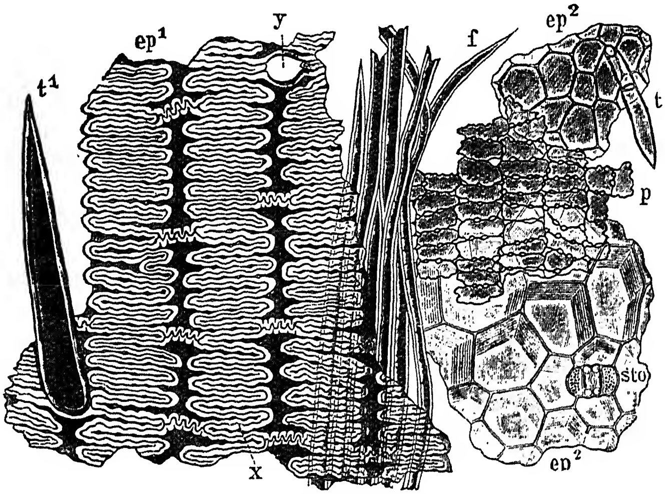

FIG. 196.—Rice husk, surface view of tissues. *ep*^{1}, outer epidermis; *ep*^{2}, inner epidermis; *f*, fibrous hypoderma; *p*, parenchyma; *sto*, stoma; *t*^{1}, *t*^{2}, hairs; *x*, deeply sinuous wall of outer epidermis cell; *y*, scar of hair. (A. L. Winton.)

(*f*) *Maize.*—The epidermis consists of polygonal pitted cells, next to which is a layer of similar but rather longer cells with somewhat thicker walls. These are followed by fibrous cells with strongly thickened walls which are not pitted. The tubular cells are smaller, more numerous and closer together than they are in wheat.

The spindle and paleæ are characterised by the abundance of pitted cells with more or less strongly thickened and lignified walls.

II.—Oil Cake

(*a*) *Linseed.*—See p. 206.

(*b*) *Almond.*—See p. 240.

(*c*) *Ground Nut.*—The pressed seeds of *Arachis hypogæa*, L. The cake is sharply characterised by the epidermal cells of the seed coat which, in surface view, exhibit a remarkable fringe-like thickening; transverse sections show that this occurs on the outer wall only. The inner layers form a stellate parenchyma with large intercellular spaces. The cells of the cotyledons contain small starch grains in addition to aleurone grains and oil. The pericarps, which are sometimes present, contain abundant sclerenchymatous fibres often strongly indented by other fibres that cross them transversely.

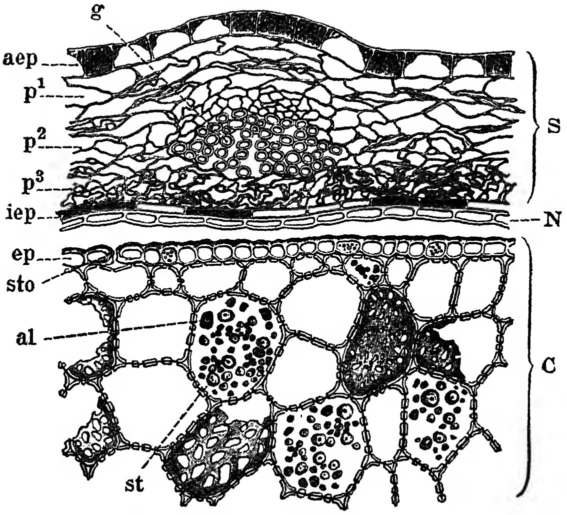

FIG. 197.—Ground Nut, transverse section of seed. *S*, Seed coats; *aep*, outer epidermis; p^1, p^2, p^3, parenchyma; *g*, fibrovascular bundle; *iep*, inner epidermis; *N*, remains of endosperm; *C*, cotyledon; *ep*, epidermis; *st*, starch; *sto*, stoma; *al*, aleurone grains. (A. L. Winton.)

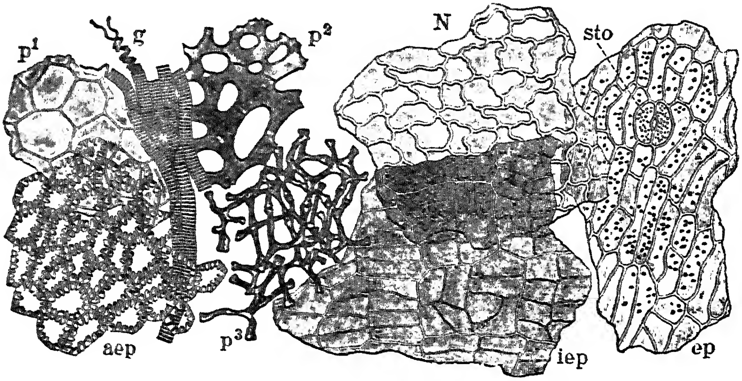

FIG. 198.—Ground Nut, surface view of tissues; *aep*, outer epidermis of seed coat; *ep*, epidermis of cotyledon; *g*, fibrovascular bundle; *iep*, inner epidermis of seed coat; *N*, remains of endosperm; p^1, p^2, p^3, parenchyma of seed coat; *sto*, stoma. (A. L. Winton.)

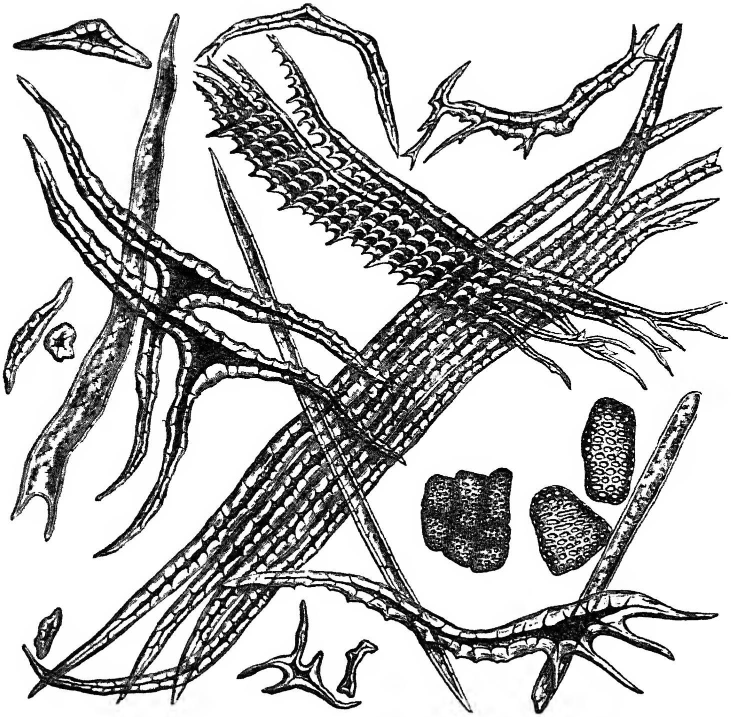

FIG. 199.—Ground Nut, isolated elements of pericarp. (Moeller.)

(*d*) *Rape.*—The structure closely resembles that of mustard seed, from which, however, it may be distinguished chiefly by the sclerenchymatous cells, which are reddish-brown in colour and larger than those of mustard, and have radial walls thickened throughout nearly the whole of their length.

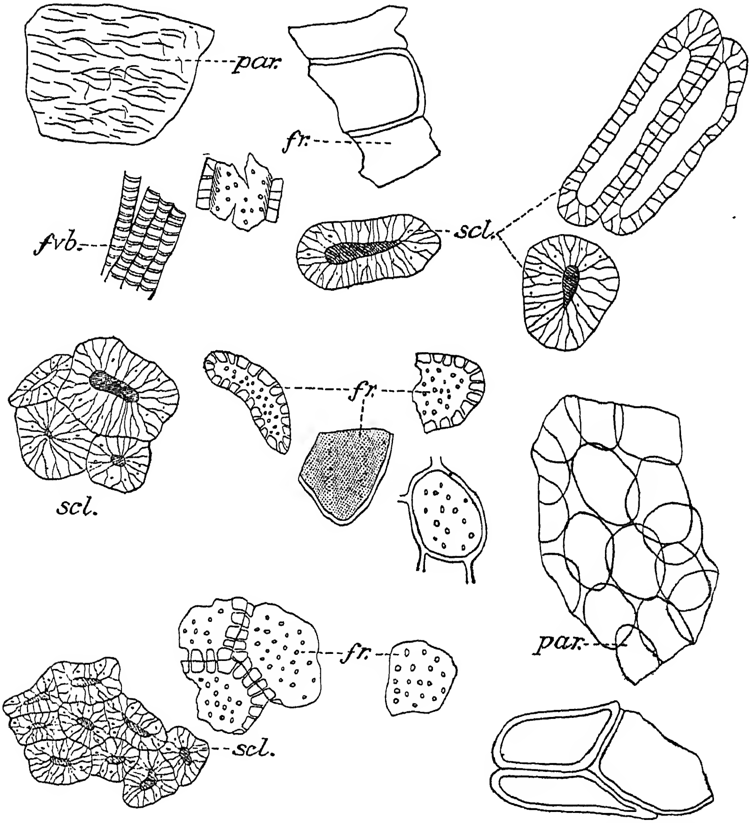

FIG. 200.—Powdered Almond shells. *scl.*, sclerenchymatous cells; *fr.*, fragments of same; *fvb.*, portion of fibrovascular bundle; *par.*, parenchymatous tissue. ×240.

III.—Nut and other Shells

(*a*) *Almond Shells.*—The powdered shells consist of sclerenchymatous cells varying considerably in size, shape and thickness of wall. Most of the cells vary in colour from yellow to yellowish-brown, and are nearly isodiametric, with moderately thickened walls, but some are nearly colourless and others dark yellowish-brown. They occur in small masses or they may be more or less isolated or even broken into fragments, according to the extent to which pulverisation has been carried; the fragments show a distinctly pitted surface. Fragments of fibrovascular bundles or of yellowish-brown thin-walled parenchymatous cells are fairly frequent. The characteristic features are the pervading yellowish-brown colour and the great variety in the thickness of the cell wall. Powdered almond shells may be distinguished from powdered cocoa-nut shells by their brighter colour, by the numerous isodiametric cells, and by the dark brown contents of those that are elongated. The cells of olive stones are elongated, nearly colourless and very strongly thickened. Neither cocoa-nut shells nor olive stones in powder contain fragments of comparatively thin-walled cells exhibiting a pitted surface.

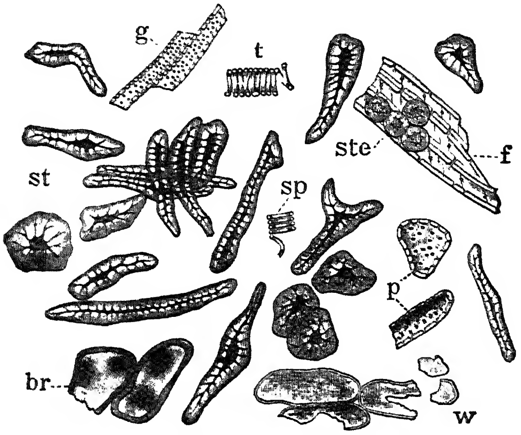

Fig. 201.—Powdered cocoa-nut shells. *br*, brown parenchymatous tissue; *f*, bast fibres; *g*, pitted vessel; *p*, parenchymatous tissue of seed coat; *sp*, spiral vessel; *ste*, stegmata; *st*, dark, yellowish-brown sclerenchymatous cells; *t*, reticulated vessel; *w*, colourless parenchymatous tissue. (A. L. Winton.)

(*b*) *Cocoa-nut Shells.*—The sclerenchymatous cells of which cocoa-nut shells consist are mostly elongated and have very thick, strongly pitted, yellowish-brown to dark-brown walls and brown contents; a few have nearly colourless walls. Fragments of brown thin-walled parenchymatous tissue and of fibrovascular bundles are also to be found. Minute, rounded, tuberculate nodules of silica contained in the stegmata (compare p. 33) occur free in the powder, but are not readily detected.

(*c*) *Hazel-nut Shells*.—Most of the cells are comparatively small (10 to 75 μ long, up to 25 μ wide) and nearly isodiametric or distinctly elongated; some are strongly elongated (150 μ long, 15 μ wide). Many have rather large cavities and colourless or pale yellowish walls with numerous branching pits; the contents are a scanty brownish substance reacting for tannin. Characteristic for this powder is the occasional occurrence of a long (150 to 250 μ) tapering, thick-walled hair derived from the epidermis.

(*d*) *Walnut Shells*.—Some of the cells are small, nearly isodiametric and strongly thickened; others are larger and have moderately thick, somewhat wavy walls.

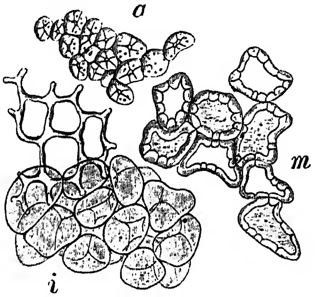

FIG. 202.—Walnut shells. *a*, sclerenchymatous cells of outer part; *m*, of central part; *i*, brown, parenchymatous tissue of inner part. (Moeller.)

Fragments of brownish, thin-walled parenchyma are fairly numerous. The powder is distinguished from that of almond shells by the absence of the bright yellowish-brown colour, from that of cocoa-nut shells by the absence of the dark-brown colour, from that of olive stones by the absence of the strongly elongated cells that are the typical feature of the latter.

IV.—Seeds

(*a*) *Olive stones.*—These are composed chiefly of sclerenchymatous cells. The typical form is a rather strongly elongated colourless cell with walls so thick that the cavity is reduced to a line; these walls are usually finely striated, they exhibit numerous branching pits, and are seldom quite straight. Occasionally fragments of the epidermis of the seed with swollen walls (fig. 203, *ep.*, clearly seen after warming in solution of chloral hydrate), of the inner layers of the mesocarp, and even of the outer epidermis of the pericarp may be found.

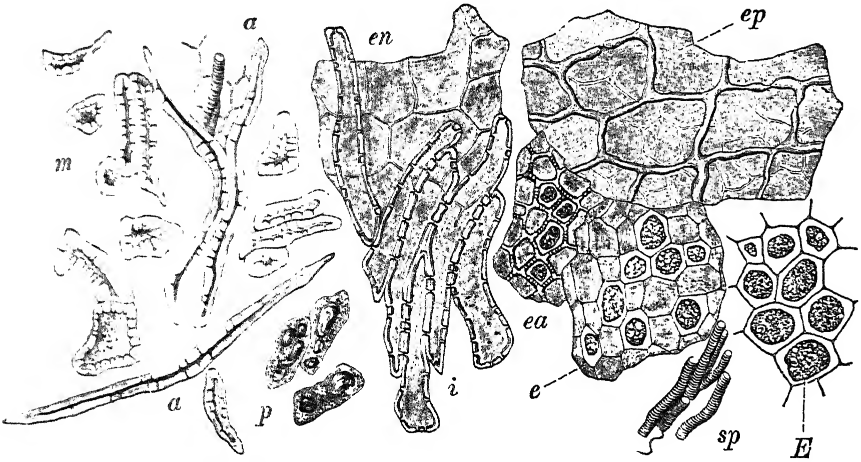

FIG. 203.—Olive stones. *a*, *m*, and *i*, sclerenchymatous cells of outer, middle and inner portion of endocarp; *E*, *e*, parenchyma of cotyledon; *ea*, outer portion of endosperm; *en*, tissue lining endocarp; *ep*, epidermis of seed coat; *p*, parenchyma of pericarp, with oil globules; *sp*, spiral vessels. (Moeller.)

(*b*) *Date stones.*—These consist of the seeds enclosed in the seed coats. The epidermis of the outer seed coat is composed of elongated cells with thickened, distinctly pitted walls. In the parenchymatous tissue below the epidermis large, thin-walled, more or less isolated cells with conspicuous reddish contents occur. The greater part of the stone, however, consists of the endosperm, the cells of which have strongly thickened (average 15 μ) walls with numerous, large, characteristic pits slightly enlarged near the middle lamella. The cell walls stain slowly blue with chlorzinciodine. The cells contain oil and proteids.

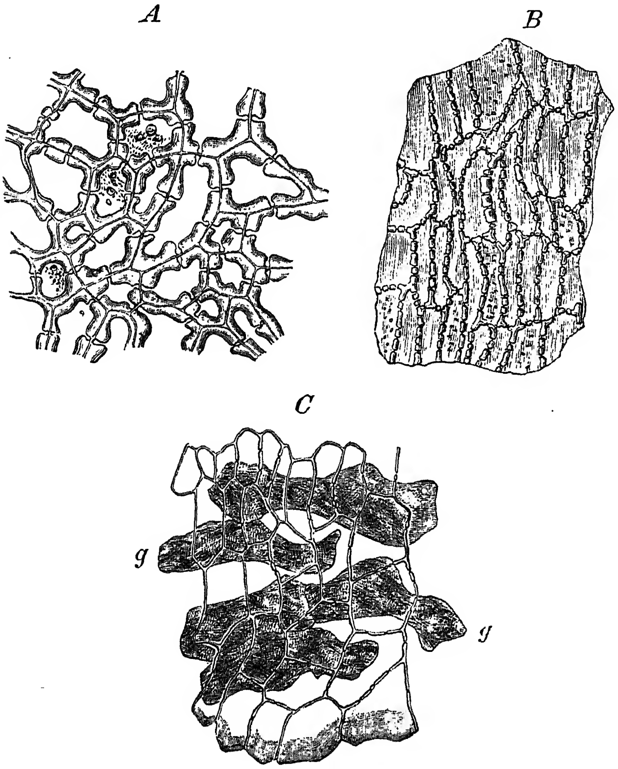

FIG. 204.—Date stones. *A*, endosperm; *B*, epidermis of seed coat; *C*, parenchymatous tissue of seed coat with pigment cells, *g*. (Moeller.)

(*c*) *Acorns.*—The chief characteristic of acorn meal is the starch, the grains of which are mostly elongated and have a large central hilum. The parenchymatous cells are large and thin-walled. Occasional long, thin-walled, sinuous hairs (from the seed coat) may be met with.

(*d*) *Cocoa-nut.*—The kernel of the cocoa-nut consists of a copious, white endosperm surrounded by a thin

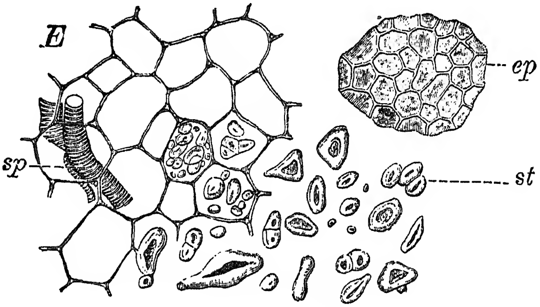

FIG. 205.—Acorn, tissues of cotyledon. *E*, parenchyma; *ep*, epidermis; *sp*, spiral vessels; *st*, starch. (Moeller.)

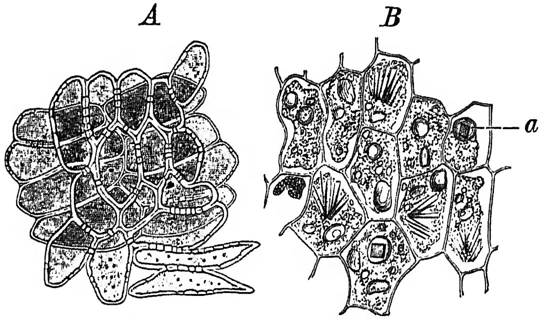

FIG. 206.—Cocoa-nut, tissues of seed. *A*, parenchymatous tissue of seed coats; *B*, cells of endosperm; *a*, aleurone grains. (Moeller.)

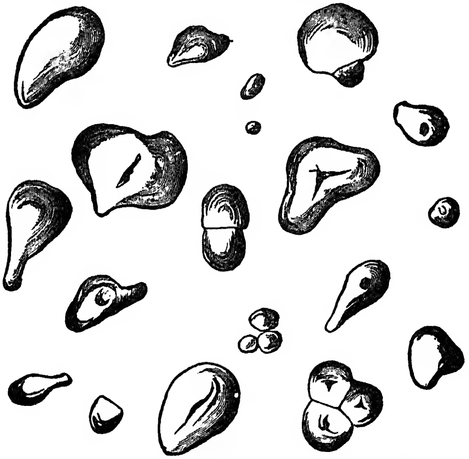

FIG. 207.—Chestnut starch. ×600. (Moeller.)

brownish seed coat. The cells of the endosperm are polygonal and have straight or slightly wavy walls; they contain crystals of fat and large aleurone grains with distinct crystalloids. The seed coats are composed largely of brownish parenchymatous tissue traversed by fibrovascular bundles which are accompanied by sclerenchymatous cells with moderately thick pitted walls.

(*c*) *Chestnuts* (*Castanea vesca*, L.).—The cotyledons consist of large, thin-walled, parenchymatous cells filled with characteristic starch grains.

V.—Woods

(*a*) *Coniferous Wood.*—Fragments of coniferous wood are readily identified, as the tracheids of which they are chiefly composed bear characteristic, large, areolated pits which, however, are seen in outline when the radial wall only of the tracheid is presented to the observer; when the tangential wall is exhibited, the pits are seen in section (fig. 208). As sawdust is commonly used to clean the drug mills, it is by no means uncommon to find small, isolated fragments of pine wood in powdered drugs.

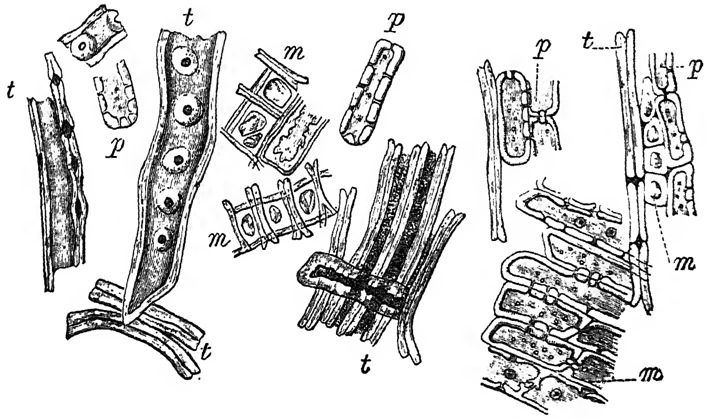

FIG. 208.—Coniferous sawdust. *m*, cells of medullary ray; *p*, parenchymatous cells; *t*, tracheids. (Moeller.)

(*b*) *Angiospermous Wood.*—This usually consists of wood fibres, vessels, parenchyma and medullary rays.

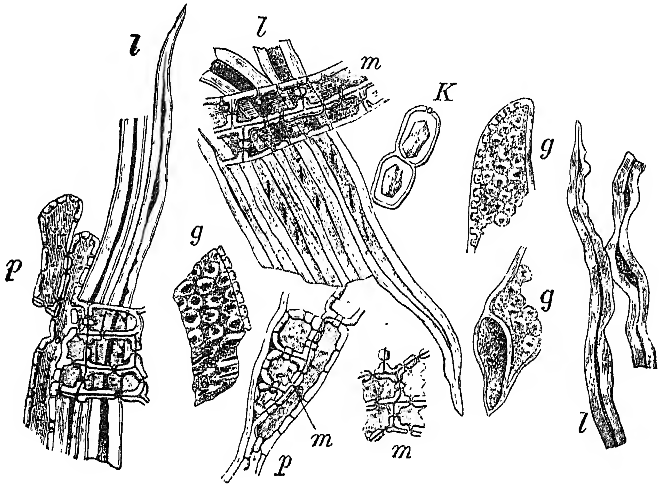

FIG. 209.—Angiospermous sawdust. *g*, fragments of vessels; *K*, cells with calcium oxalate; *l*, sclerenchymatous fibres; *m*, cells of medullary ray; *p*, wood parenchyma. (Moeller.)

The fibres usually occur in irregular masses, comparatively free from calcium oxalate cells, but often accompanied by lignified medullary rays exhibiting their radial or tangential section; these characters suffice to distinguish wood fibres from grouped bast fibres. The structure of the many angiospermous woods used for technical purposes is so uniform, that it is exceedingly difficult to determine the botanical origin of a powdered wood that may be found as a sophistication in vegetable powders. The identification of such woods as are employed medicinally is comparatively easy.

SECTION XV

GENERAL SCHEME FOR THE EXAMINATION OF POWDERS

The following outline may serve as a guide to the systematic examination of an unknown powder. In each case the material should be carefully bulked, either by shaking in a bottle, or by triturating in a mortar or, if in small quantity, by mixing on paper with a spatula. From 1 to 2 grammes should then be set aside for use.

Examination

Fine Powders.—Thoroughly mix with a glass rod in a watch-glass or hollowed glass block 0·1 gramme to a thin cream with (*a*) water, (*b*) dilute glycerin, and (*c*) solution of chloral hydrate. Cover (*a*) and (*c*) closely but (*b*) only loosely with a small filter paper, so that the glycerin may slowly become more concentrated. Allow these to stand about twelve hours. Then stir again with a glass rod and mount a little of each, taking about as much as a white mustard seed in volume, diluting with the medium in which it has been soaked, and covering with a ¾-inch square coverslip.

(*a*) Examine the water preparation under the low power (50 to 70 diameters). Study first the single cells, especially those which are well preserved, such as sclerenchymatous cells, bast fibres, &c., then groups of a few cells and fragments of cells, making them roll, if necessary, by touching the edge of the coverslip with a dissecting needle. Use the high power (300 to 400 diameters) for the examination of details. Continue the study of each cell, or group of cells, until its structure is thoroughly understood, and make sketches of what is seen. Should the identity of the powder be known, compare these sketches with published illustrations or with similar sketches made from the genuine powders or from sections of the original substance. Keep the slide for future reference, protecting it from evaporation.

Examine for the following cell contents:

(α) *Starch.* Determine its presence or absence by the iodine reaction (p. 9). If present, examine, measure, and sketch it. If present in large quantity, remove it (pp. 23, 299, 312), and examine the residue.

(β) *Oil.* Determine its presence or absence by the alkanna reaction (p. 38). If present in large quantity, defat (p. 202), and examine the residue.

(γ) *Aleurone Grains.* Determine their presence or absence by the iodine or picric acid reaction (p. 192). If present, examine their contents (p. 190).

(δ) *Mucilage.* Determine its presence or absence by the ruthenium red and other reactions (p. 195).

(ε) *Calcium Oxalate.* Determine its presence or absence, using the polariser if necessary. Confirm by the acetic acid and sulphuric acid reactions.

(*b*) Examine next the glycerin preparation. The particles will be clearer than they were in water. Study the smaller and medium-sized groups of cells, and observe particularly their colour.

(*c*) Examine the powder macerated with solution of chloral hydrate. Starch (if present in the original powder) and much of the dried protoplasm, aleurone grains, &c., will have been dissolved. Study particularly the larger fragments, focussing the uppermost layer of cells first, and proceeding slowly through the fragment. Endeavour to realise the nature and arrangement of the cells, and so construct a mental image of the fragment. Fragments that are lying obliquely are often difficult to understand, but may be induced to present a more favourable aspect by being gently moved.

(*d*) If the fragments are difficult to understand, mount a little of the original powder in solution of potash (10 to 20 per cent.), cover, warm gently, cool, and crush the fragments by pressing and sliding the coverslip. Resort if necessary to a bleached preparation (see below). Examine the chloral hydrate preparation for crystals of calcium oxalate.

(*e*) If the powder is dark in colour bleach 0·5 gramme (p. 177). Examine in dilute glycerin as directed under (*b*). Stain a portion with phloroglucin and hydrochloric acid (p. 62) for lignified tissues, and another with corallin soda for mucilage and callus plates.

(*f*) Mount a fresh portion in a mixture of two volumes of castor oil and one of absolute alcohol, or in liquid paraffin, or olive oil. Compare this preparation with the one in water to ascertain if any water-soluble substances have been removed by that mountant.

(*g*) If much sclerenchymatous tissue is present, disintegrate about 0·5 gramme with potassium chlorate and nitric acid (p. 56), collect the powder in a centrifuge tube, wash and examine.

If time does not allow of the preparations (*a*), (*b*), and (*c*) being allowed to stand for twelve hours, proceed as follows :

(*a*) Moisten a little of the powder on a slide with alcohol, allow it to stand until most of the alcohol has evaporated, mix with water, cover and examine.

(*b*) Thoroughly mix a little with water on a slide, using a small glass rod as a stirrer ; allow it to stand a few minutes, add a drop of glycerin, and examine.

(*c*) Moisten with alcohol on a slide, add solution of chloral hydrate, cover, warm gently until the solution just commences to boil, cool, and examine.

Coarse Powders.—Sift out that which will pass through a No. 60 or 80 sieve. Examine this as directed for fine powders.

Examine with a lens that which does not pass through the sieve, pick out if possible fragments sufficiently large and cut sections from them.

Mount a few fragments in 20 per cent. solution of potash, cover, gently warm, cool, crush the fragments, and examine. Or tease them out with the needles after warming with solution of potash.

Powder the coarser fragments in a mortar until they too pass through the sieve ; mix all the powder carefully and proceed as directed for fine powders.

Double Staining of Powders.—Although staining may generally be dispensed with in the examination of powders, very excellent preparations may be obtained if desired by the following (Cordonnier's [1]) method :

(*a*) *Non-fatty Powders.*—Introduce into a centrifuge tube 5 c.c. of solution of chlorinated soda and about 0·1 gramme of the bulked powder. Shake the mixture occasionally during ten minutes, then dilute with distilled water, and centrifuge until the powder is collected at the tip of the tube. Decant the supernatant liquid, diffuse the residue in water and centrifuge again, repeating the process until the hypochlorite is eliminated. Decant the last washings and add 5 c.c. of the combined reagent (see below). Leave the powder in contact with the stain for eight to ten minutes, dilute with water and wash several times with the aid of the centrifuge. Then pass the powder successively through 60 per cent. alcohol, 90 per cent. alcohol, absolute alcohol (twice) and xylol, decanting after each washing. Finally mix the stained powder well, place a drop of Canada balsam on a slide, add a drop of the stained magma, mix, cover, press gently and allow to dry.

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[1] *Bulletin des Sciences Pharmacologiques*, 1903, p. 21.

(*b*) *Fatty Powder.*—Defat the powder by two or three successive treatments with xylol, then decolourise with solution of chlorinated soda and proceed as above.

If the powder is rather coarse, prolong the action of each liquid correspondingly.

Combined Reagent for Double Staining:

Introduce into a flask capable of holding 1200 cubic centimetres in the order named: Iodine green (Grübler), 1 gramme; chloroform, 10 grammes; alum-carmine, 1000 cubic centimetres. Shake till dissolved and filter.

Make the alum-carmine as follows: Treat 1 gramme of carmine with 5 grammes of alum and a little distilled water. Evaporate to dryness at a gentle heat. Allow the residue to stand for twenty-four hours, dissolve it in 100 cubic centimetres of cold, distilled water, and filter.

Determination of the Origin

The following procedure may be adopted to determine the organ from which a powder has been derived:

(*a*) Observe the colour of the particles as seen in dilute glycerin. A green colour indicates leaf, leaf-stalk, herbaceous stem, or, possibly, calyx of a flower.

Examine the chloral hydrate preparation. The presence of an epidermis with stomata and polygonal or wavy cells, of branching veinlets and of palisade or spongy parenchyma, indicates a leaf. Elongated, rectangular epidermal cells are probably derived from the midrib, or from an herbaceous stem; fragments consisting of large, elongated, colourless parenchymatous cells point to the former. If pollen grains are found, a flower may be suspected, and search should be made for portions of the petal, which will probably be coloured and have a papillose epidermis, and for the characteristic, spirally or reticulately thickened cells from the endothecium of the anthers.

(*b*) If chlorophyll is absent, examine the chloral hydrate preparation for vessels. In the absence of chlorophyll these will indicate the presence of wood, which may be derived from a trunk, a root, or a rhizome. Abundant, irregular fragments, consisting of wood fibres with medullary rays crossing them at right angles and with comparatively little calcium oxalate, indicate a wood. On the other hand, abundant parenchymatous tissue, filled with starch, oil, or other reserve material, indicates a root or rhizome; in this case, sclerenchymatous cells, or bast fibres, isolated, or in more or less regular groups, may be present.

(*c*) If vessels are absent, stain a bleached preparation with corallin soda (see list of reagents), and examine for large sieve tubes. These, in conjunction with fragments of cork, and possibly with isolated or grouped bast fibres and sclerenchymatous cells, indicate a bark.

(*d*) If the powder is free from chlorophyll, large vessels and sieve tubes, it is probably derived from a seed or fruit. Examine for aleurone grains, the presence of which is definite evidence of a seed, and examine also for parenchymatous tissue with other reserve material such as starch, oil, cellulose, &c. The presence of an epidermis with more or less distorted stomata, of the so-called aleurone layer, or of much empty parenchymatous tissue indicates a fruit.

Identification of the Powder

Having determined the organ from which an unknown powder is derived, the next step is its definite identification. This demands considerable skill and experience. It is best effected by comparing the sketches made of the tissues and elements of the powder, with illustrations published in the various works dealing with this subject.[1]

The determination should invariably be confirmed by powdering the substance indicated and comparing the two powders under similar conditions.

Determination of Purity

The microscopical examination of a vegetable powder often has for its object the determination of the purity or otherwise of a powder of given origin. In such case, the comparison with a specimen of the powder of about the same degree of fineness and known to be genuine is absolutely necessary. Care must also be taken to interpret correctly the results of the examination.

The methods adopted for the sophistication of powders may be classed under the following heads:

1. Total substitution of one substance for another.
2. Use of a substance of inferior quality.
3. Intentional addition.
4. Intentional abstraction.
5. Intentional abstraction and addition.

1. *Total Substitution.*—This is, as a rule, easily detected, although in some cases—as, for instance, the substitution of powdered cassia for powdered cinnamon—considerable caution has to be exercised.

2. *Quality.*—The determination of the relative quality of a genuine powder is obviously in the first instance to be effected by chemical analysis. Nevertheless, the microscopical examination may give valuable assistance and may

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[1] Greenish and Collin, *Anatomical Atlas of Vegetable Powders*; Winton, *The Microscopy of Vegetable Foods*; Schneider, *Powdered Vegetable Drugs*; Vogl, *Die Wichtigsten Vegetabilischen Nahrungs- und Genussmittel*; Solereder, *Systematic Anatomy of the Dicotyledons.*

be successful where chemical analysis fails. The colour of the powder, the contents of the cells, especially of such as contain active constituents, the numbers in which such cells occur, and the presence of tissues not found in good samples, possibly the result of careless collection, may give useful indications of the quality of the powder.

3. *Intentional Addition.*—Not unfrequently foreign substances accidentally find their way in very small quantities into vegetable powders, and caution is necessary not to consider such as cases of adulteration. Drug mills are commonly cleaned with sawdust, and an occasional tracheid of pine wood may be detected in most powdered drugs. Only when the quantity is considerable can this be considered serious. It is practically impossible to collect rhizomes without, in some cases at least, portions of the aerial stems or even leaves; hence the tissues of these organs may find their way into the powders without constituting a sophistication. Barks often have portions of wood adhering to them, stems portions of bark; leaves are accompanied by the smaller stems, by flowers and occasionally small fruits.

The quantity in which such foreign organs are present is best judged by comparison with powders containing varying, known percentages of them. Intentional addition, however, is usually carried to such an extent as to leave little doubt of the fraudulent intention. Comparison of the suspected powder with one known to be genuine is indispensable.

4. *Intentional Abstraction.*—Sophistication of this nature generally takes the form of adulteration of the genuine powder with the powder of the same drug previously deprived of its active constituents; thus powdered ginger is occasionally adulterated with exhausted or 'spent' ginger. Such sophistication is best detected by chemical means, although indications of it may be obtained by means of the microscope.

5. *Intentional Abstraction and Addition.*—Here the deficiency in taste, odour or colour, caused by admixture with exhausted powder, is cloaked by the addition of some foreign material. The loss of bitterness of powdered gentian due to such cause has been concealed by the addition of powdered quassia, a sophistication which may be readily detected.

APPENDIX A

REAGENTS OF GENERAL UTILITY

The following list comprises the reagents mentioned in the foregoing pages, together with a few others that are occasionally employed. It makes, however, no pretensions to being exhaustive; on the contrary, numerous reagents that have been recommended from time to time for various special purposes, for which doubtless they are useful, have been omitted as being unnecessary for the student. This is especially the case with the host of staining reagents, of which but few have been retained as specially useful for particular purposes. Each reagent has been described, so that the student may either prepare it himself or easily procure it, and to most of them notes have been appended indicating the uses to which they are generally put. The student is strongly advised to refrain from the indiscriminate use of staining reagents. Each experiment should be designed to obtain certain definite information with regard to the preparation under examination.

Acetic Acid.—Containing 33 per cent. of real acetic acid; the Acidum Aceticum of the British Pharmacopœia; it is used for distinguishing between calcium oxalate, which is insoluble, and calcium carbonate, which dissolves with effervescence; for neutralising an excess of caustic potash, and other purposes.

Acetic Acid, Dilute.—The Acidum Aceticum Dilutum of the British Pharmacopœia, containing 4 per cent. of real acetic acid.

Alcohol.—Absolute alcohol is to be preferred, but for many purposes methylated spirit made with wood naphtha can be used; the ordinary methylated spirit made with mineral naphtha is of limited use only, since it makes a turbid mixture with water.

Alcohol is employed for a great variety of purposes. It removes air from sections of dried drugs, dissolves resin, volatile oil, tannin, chlorophyll, &c.; it dissolves most fixed oils in small but appreciable quantity, castor oil freely. Gum and inulin are quite insoluble in it, certain sugars are only slightly soluble.

Alcohol, 90 per cent., is also frequently used, and may be often employed in the place of absolute alcohol; it has less solvent action upon fixed oils.

Alkanna, Tincture of.—

Alkanet root	20 grammes.
Alcohol, 90 per cent. . . .	100 cubic centimetres.

Macerate for a week, and filter.

Tincture of alkanna is much used as a staining agent for fixed oils. For this purpose it should be diluted with an equal volume of water immediately before use, and sections left immersed in it for several hours. No fixed oil or fat is known which will not, under these conditions, assume a red colour; but, on the other hand, other substances, such as resin, caoutchouc, &c., may also yield the reaction.

Ammonia, Dilute.—The Liquor Ammoniæ of the British Pharmacopœia, containing 10 per cent. by weight of ammonia gas.

Ammonia, Strong.—The Liquor Ammoniæ Fortis of the British Pharmacopœia, containing 32·5 per cent. by weight of ammonia gas. It is used for the preparation of cuoxam.

Aniline Chloride.—A saturated solution in water is sometimes used to stain lignified cell walls, to which it imparts a golden yellow colour. It is, however, inferior in this respect to phloroglucin, and hence is seldom used. It acts better when acidified with hydrochloric acid.

Bismarck Brown.—A very dilute aqueous solution, about the colour of brown sherry, is useful to stain elements after separation by potassium chlorate and nitric acid, or by chromic acid, since treatment with these reagents renders the tissues very transparent. (Compare also safranin.)

A saturated aqueous solution, filtered, is occasionally used as a stain for mucilage.

Braemer's Reagent.—

Sodium tungstate . . .	1 gramme.
Sodium acetate	2 grammes.
Water to make	10 cubic centimetres.

Dissolve. This is one of the best reagents for tannin, with which it produces a yellowish-brown precipitate. While iron salts are commonly used to detect tannin, it must be remembered that a number of other plant constituents give dark-greenish or bluish-black colorations with this metal; hence, although a negative result is decisive, a positive result does not necessarily indicate tannin. In such cases confirmation is obtained by Braemer's reagent.

The section to be tested, which should not have been treated with any solvent of tannin, is immersed in a drop of the reagent on the slide; as the reagent penetrates, a characteristic yellowish-brown or reddish-brown precipitate is produced.

Chloral Hydrate, Solution of.—

Chloral hydrate	50 grammes.
Water	20 cubic centimetres.

Dissolve. The solution is a most valuable clearing agent. It not only induces expansion of cells that have shrunk during the drying of the drug, but also dissolves many of the commoner constituents such as chlorophyll, resin, volatile oil, proteid matter, starch, &c.

Chloral Iodine.—The foregoing solution saturated with iodine, a few crystals of which should be kept in it. It is useful for the detection of minute starch grains.

Chlorinated Soda, Solution of (Meyer).—

Chlorinated lime . . .	200 grammes.
Distilled water	1,750 grammes.

Triturate the chlorinated lime with the water, added gradually; transfer to a stoppered bottle, and add—

Sodium carbonate . . .	250 grammes,

dissolved in

Distilled water	750 grammes.

Shake together for four days, keeping the bottle protected from light; filter; to the filtrate add a 10 per cent. solution of potassium oxalate as long as a precipitate forms; stand, and filter.

Solution of chlorinated soda is extremely useful for bleaching sections and preparations, the colour of which is too dark to allow of the details being clearly seen. The bleaching should not be continued longer than necessary; when completed, the preparations should be washed with water.

The reagent should be kept protected from light.

Chlorzinciodine, Solution of.—

Solution of zinc chloride, sp. gr. 1·8	100·00 grammes.
Potassium iodide . . .	10·00 grammes.
Iodine	0·15 gramme.

Dissolve the potassium iodide and the iodine in 10 cubic centimetres of water; add this to the solution of zinc chloride; stand until bright. Keep a few crystals of iodine in the solution.

Solution of zinc chloride, B.P. .	175·0 cubic centimetres.
Potassium iodide . . .	20·0 grammes.
Iodine	0·5 gramme.
Distilled water	15·0 cubic centimetres.

The following method also yields good results:

Evaporate the solution of zinc chloride to 100 cubic centimetres;

to the solution, while still hot, add the potassium iodide and iodine, previously dissolved in the water; let the mixture stand till cold.

Very commonly used for the differentiation of cellulose from lignified walls. The section should be mounted in a drop of water on a slide, the water *completely* removed by filter paper, and a drop of the reagent dropped on to it. Cellulose walls are (often slowly) coloured blue or violet, lignified and suberised walls yellow or brown; starch grains swell, and are coloured blue.

Chromic Acid, Solution of.—

Chromic acid	10 grammes.
Dilute sulphuric acid (containing 10 to 15 per cent. of sulphuric acid)	90 cubic centimetres.

Dissolve. The reagent is useful for separating sections into their constituent cells. Several sections are immersed in the reagent in a watch-glass, and one from time to time (about every fifteen minutes) removed, washed with a drop of water, and gently pressed with a glass rod. When, under this treatment, the constituent cells separate readily from one another, the remainder of the sections are washed with water and transferred to alcohol until required.

Long-continued action of the reagent results in the destruction and solution of the cellulose and lignified cell walls. Suberised walls resist its action much longer.

Corallin Soda, Solution of.—

Sodium carbonate	30 grammes.
Distilled water	70 grammes.

Dissolve. To a little of this solution add a small fragment of corallin, or sufficient alcoholic solution of corallin to produce a bright pink colour. The mixture must be freshly prepared.

The special use to which corallin soda is put is the staining of callus plates and the detection of sieve tubes by this means. The solution should be of a pale bright pink colour (not dark wine red) and should be freshly prepared, as in this dilution it rapidly loses its staining power. The reagent also imparts a reddish colour to lignified tissue, starch grains, and some forms of mucilage.

Cuoxam.—This reagent must be freshly prepared with strong solution of ammonia. The following (Vogl's) method is a convenient one:

Prepare some cupric oxycarbonate by precipitating a solution of cupric sulphate with sodium carbonate; wash the precipitate first by decantation, afterwards on the filter until free from sulphate. Drain well and dry by exposure to the air. Keep the dry powder in a stoppered bottle. When the reagent is required, dissolve a little in strong solution of ammonia.

It dissolves cellulose.

Eosin.—A dilute solution in water is occasionally useful for staining cell contents, especially aleurone grains.

Fehling's Solution.—

Cupric sulphate	34·6 grammes.
Distilled water to make . .	500·0 cubic centimetres.

Dissolve.

Sodio-potassium tartrate . .	173·0 grammes.
Potassium hydrate . . .	125·0 grammes.
Distilled water to make . .	500·0 cubic centimetres.

Dissolve. Mix equal volumes of each solution for use when required. The reagent is used to detect reducing sugars, with which it yields a red precipitate of cuprous oxide; with some proteids a bluish or reddish-violet coloration is produced.

Ferric Chloride, Solution of.—A 1 per cent. solution of ferric chloride in distilled water. Frequently used as a reagent for tannin. See also Braemer's Reagent.

Glycerin.—Pure glycerin of sp. gr. 1·260.

Glycerin, Dilute.—Pure glycerin diluted with an equal volume of distilled water.

Both of these are very largely used as mounting media.

Gum and Glycerin.—

White gum acacia . . .	40·0 grammes.
Water	30·0 cubic centimetres.
Glycerin	40·0 grammes.
Thymol	0·1 gramme.

Dissolve. Used for fixing small seeds, &c., on pith. The glycerin prevents the gum from being hard when dried.

Hydrochloric Acid.—Pure hydrochloric acid of sp. gr. 1·16. Used in conjunction with phloroglucin for detecting lignification. Diluted with an equal volume of water for dissolving calcium oxalate.

Iodine Water.—Distilled water saturated with iodine, a few crystals of which should be kept in the reagent. Protect it from the action of light by keeping it in the dark or in amber-coloured bottles. Used as a reagent for starch and for aleurone grains. Solution of iodopotassium iodide, diluted with water to the colour of brown sherry, is often used in its place.

Iodopotassium Iodide, Solution of.—

Iodine	2 grammes.
Potassium iodide	1 gramme.
Distilled water	200 cubic centimetres.

Dissolve. The reagent, which may be diluted with water if necessary, stains proteid matter yellow, starch blue, suberised and lignified walls yellow. The cellulose cell wall assumes a yellow colour, which is changed to blue by irrigation with concentrated sulphuric acid.

Maceration Mixture, Schulze's.—Potassium chlorate and nitric acid; the strength of the latter may be varied to suit the requirements of the case; an acid of sp. gr. 1·3 is very generally useful.

The reagent is well adapted for the separation of the elements of woody tissues from one another, and also for the destruction of the more delicate parenchymatous cells and their removal from powdered drugs.

Methylene Blue, Alcoholic Solution of.—

Methylene Blue	0·1 gramme.
Alcohol (95 per cent.)	25·0 cubic centimetres.

Dissolve.

Methylene Blue, Glycerin Solution of.—

Methylene Blue	0·2 gramme.
Alcohol (95 per cent.)	10·0 cubic centimetres.
Glycerin	40·0 cubic centimetres.

Dissolve. Useful for staining mucilage (compare p. 118).

Millon's Reagent.—

Mercury	3 cubic centimetres.
Fuming nitric acid	27 cubic centimetres.

Dissolve without heat; dilute the solution with an equal volume of water. Proteid matter in contact with Millon's reagent gradually assumes a bright brick-red colour. As the activity is liable to diminish by long keeping, it should be tested on a section known to contain proteid matter before it is used as a reagent.

Naphthol Solution.—

α-Naphthol	10 grammes.
Alcohol	100 cubic centimetres.

Dissolve. Gives, in conjunction with sulphuric acid, a violet coloration with inulin. Allow a drop of the reagent to remain on the section for a minute or two and remove with filter paper; drop on two or three drops of concentrated sulphuric acid, cover, and warm gently. An intense violet coloration is produced if inulin is present.

Osmic Acid, Solution of.—A 1 per cent. aqueous solution of osmic acid. It should be protected from light. Osmic acid gradually colours fixed oils dark brown or nearly black. It does not, however, react with all fats, palmitin, stearin, and certain others not being coloured by it.

Phloroglucin, Solution of.—

Phloroglucin	1 gramme.
Alcohol (90 per cent.) . . .	100 cubic centimetres.

Dissolve. It gradually darkens in colour, and at the same time loses its power. It should not be kept more than three months. The section to be tested should be immersed in a few drops of the reagent for five minutes, the excess removed with filter paper, and a drop of strong hydrochloric acid added. Lignified cell walls are stained pale to dark red, according to the degree of lignification.

The reagent is sometimes made by dissolving phloroglucin in alcohol and adding hydrochloric acid.

Picric Acid, Solution of.—A saturated aqueous solution is used to stain aleurone grains yellow.

Potash, Solution of.—A 5 per cent. aqueous solution of potassium hydrate. Largely used as a clearing agent. It induces swelling of the cell wall and consequent expansion of dried cells ; it swells and dissolves starch, dissolves proteid matter, tannin, &c. It is also employed for disintegrating parenchymatous tissues, these being digested in the reagent, diluted if necessary with water, in a water-bath.

Potash, Ammoniacal Solution of.—Wash stick potash with water to remove the carbonate on its surface, and add water in quantity insufficient to dissolve the whole of the potash. Pour off the saturated solution and add an equal volume of strong solution of ammonia (sp. gr. 0·910).

This reagent has been recently advocated as specially useful for the identification of fixed oils. It saponifies all fatty oils, producing with non-drying oils radiating filiform crystals, and with drying oils granules. The section is immersed in the reagent, covered with a coverslip, and examined from time to time, during several hours, to ascertain the effect upon the globules to be tested.

Potash, Very Dilute Solution of.—A 0·3 per cent. solution is used to dissolve aleurone grains.

Potash, Strong Solution of.—A 20 per cent. (or even 50 per cent.) solution is used to induce swelling of refractory cell walls with a view to disclosing the structure of collapsed tissues.

Ruthenium Red, Solution of (*in Solution of Lead Acetate*).—Prepare a 10 per cent. solution of lead acetate in distilled water. To one or two cubic centimetres of this solution add enough ruthenium red to produce a wine-red colour. The solution will not keep long, and should therefore be freshly prepared.

A very useful reagent for the detection of mucilage, some varieties of which assume with it a brilliant pink coloration.

Safranin.—A very dilute aqueous solution is useful for staining colourless, transparent tissues in order to render the details more easily visible.

Soudan Glycerin.—

Soudan III.	0·01 gramme.
Alcohol (90 per cent.) . . .	5·00 cubic centimetres.

Dissolve and add—

Glycerin	5·00 cubic centimetres.

Colours the suberised wall red, especially when warmed with it, and hence is useful to detect secretion cells (the walls of which are commonly suberised) in powdered drugs. It also colours fixed and volatile oils.

Sulphovanadic Acid.—

Ammonium vanadate . . .	1 gramme.
Concentrated sulphuric acid . .	100 cubic centimetres.

Powder the ammonium vanadate and triturate it with the sulphuric acid; stand until clear. The reagent will not keep longer than a few days. It is a delicate micro-chemical reagent for strychnine.

Sulphuric Acid, Concentrated.—Pure sulphuric acid of sp. gr. 1·843. It is employed for dissolving cellulose and lignified cell walls, leaving suberised walls comparatively little affected.

Sulphuric Acid, 80 per cent.—Sulphuric acid containing 80 per cent. by weight of the pure acid may often be advantageously substituted for the above.

APPENDIX B

LIST OF THE CHIEF VARIETIES OF CELL WALL AND CELL CONTENTS, AND THE MEANS ADOPTED FOR THEIR IDENTIFICATION

(1) **Aleurone Grains.**

- (*a*) Picric acid stains them bright yellow ;
- (*b*) Iodine stains them yellowish-brown ;
- (*c*) In iodine water the crystalloid and globoid (if present) become visible ;
- (*d*) In very dilute potash they dissolve, with the exception of the globoid and calcium oxalate (if present).

(2) **Alkaloids.**—The best general reagent is solution of iodine in potassium iodide, which produces reddish-brown precipitates with almost all alkaloids, even in very dilute solutions. Sections that have been thus treated are compared with sections that have been freed from alkaloid by extraction with an alcoholic solution of tartaric acid before being submitted to the reagent. Special colour reactions, such as that of strychnine with sulphovanadic acid, often afford very valuable information. Further details must be sought in the numerous memoirs that have been published dealing specially with this subject.

(3) **Calcium Carbonate.**

- (*a*) Acetic or hydrochloric acid dissolves with effervescence ;
- (*b*) Sulphuric acid produces, in addition, acicular crystals of calcium sulphate.

(4) **Calcium Oxalate.**

- (*a*) Insoluble in acetic acid ;
- (*b*) Soluble without effervescence in hydrochloric acid ;
- (*c*) Yields acicular crystals of calcium sulphate with sulphuric acid

(5) **Callus Plate.**

(*a*) Corallin soda stains it bright pink;
(*b*) Hoffmann's blue stains it blue;
(*c*) Sulphuric acid dissolves it.

(6) **Caoutchouc.**

(*a*) Insoluble in caustic potash;
(*b*) Soluble in chloroform;
(*c*) Stains pink with tincture of alkanna.

(7) **Cell Wall, Cellulose.**

(*a*) Is stained blue or violet by chlorzinciodine;
(*b*) Is stained blue by iodine followed by sulphuric acid;
(*c*) Is not stained by aniline chloride or by phloroglucin;
(*d*) Dissolves in cuoxam.

(8) **Cell Wall, Lignified.**

(*a*) Is stained yellow or brown by chlorzinciodine;
(*b*) Is stained bright yellow by aniline chloride;
(*c*) Is stained bright red by phloroglucin and hydrochloric acid;
(*d*) Swells and dissolves in strong sulphuric acid, especially if gently warmed.

(9) **Cell Wall, Suberised.**

(*a*) Is stained yellow or brown by chlorzinciodine;
(*b*) Is stained red by Soudan red;
(*c*) Resists the action of concentrated sulphuric acid;
(*d*) Is stained yellow by strong potash; on warming, oily drops exude.

(10) **Fat.**

(*a*) In solid, often crystalline masses, which fuse to oily drops when warmed;
(*b*) These are stained by tincture of alkanna;
(*c*) Is saponified by ammoniacal potash, producing crystalline or granular soaps;
(*d*) Is soluble in ether-alcohol.

(11) **Inulin.**

(*a*) In colourless, amorphous, or sometimes sub-crystalline masses;
(*b*) Insoluble in cold water;

(*c*) Dissolves at once, without swelling, in water at 60° to 70° C.;
(*d*) Is not stained by iodine;
(*e*) Gives a violet coloration with α-naphthol and sulphuric acid.

(12) **Mucilage.**—Several varieties of mucilage are known which vary in their reactions; the following reactions are useful:
(*a*) Insoluble in alcohol and glycerin; swell and (?) dissolve in water;
(*b*) Solution of subacetate of lead colours them yellowish and makes them granular;
(*c*) May be stained by ruthenium red, corallin soda, chlorzinciodine, methylene blue or Bismarck brown.

(13) **Oil, Fixed.**
(*a*) In globules;
(*b*) Is stained pink by tincture of alkanna;
(*c*) Is stained brown by osmic acid;
(*d*) Is saponified by ammoniacal potash;
(*e*) Is soluble in ether-alcohol; not readily soluble in 90 per cent. alcohol.

(14) **Oil, Volatile.**
(*a*) In globules;
(*b*) Is stained red by tincture of alkanna;
(*c*) Does not yield soap with ammoniacal potash;
(*d*) Is soluble in 90 per cent. alcohol.

(15) **Proteid Matter.**
(*a*) Is stained yellow or brown by solution of iodine;
(*b*) Is coloured red by Millon's reagent;
(*c*) Is coloured yellow by potash after nitric acid;
(*d*) Is coloured yellow by picric acid.

(16) **Resin.**
(*a*) In irregular solid masses;
(*b*) Is stained red by tincture of alkanna;
(*c*) Is soluble in 90 per cent. alcohol.

(17) **Silica** (*if present as a visible cell content*).
(*a*) Is unacted upon by any of the ordinary reagents;
(*b*) May be recognised unaltered in the ash after treatment with hydrochloric acid.

(18) **Starch.**

(*a*) Is coloured blue by solution of iodine ;
(*b*) Is swollen by caustic potash ;
(*c*) Swells when boiled with water.

(19) **Tannin.**

(*a*) Is coloured bluish-black or greenish-black by solution of ferric chloride ;
(*b*) Gives brown or yellowish-brown precipitate with Braemer's reagent.

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